HISTORY OF THE OFFICE OF SCIENTIFIC RESEARCH AND DEVELOPMENT

A summary of the activities of the entire organization in the development of improved weapons of warfare has been published as *Scientists Against Time* by James Phinney Baxter, 3rd. Details about the different parts of the organization are presented in a series of volumes with the common title, *Science in World War II,* which has been prepared under authority from:

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James B. Conant, President, Harvard University
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Science in World War II

NEW WEAPONS FOR AIR WARFARE
  DIVISIONS 4, 5, AND 7 OF NDRC; SECTION T, OSRD

COMBAT SCIENTISTS
  OFFICE OF FIELD SERVICE; NALOC; DOLOC

ADVANCES IN MILITARY MEDICINE
  COMMITTEE ON MEDICAL RESEARCH

ROCKETS, GUNS, AND TARGETS
  DIVISIONS 1, 2, AND 3 OF NDRC

CHEMISTRY
  DIVISIONS 8, 9, 10, 11, 19 AND TDAC OF NDRC

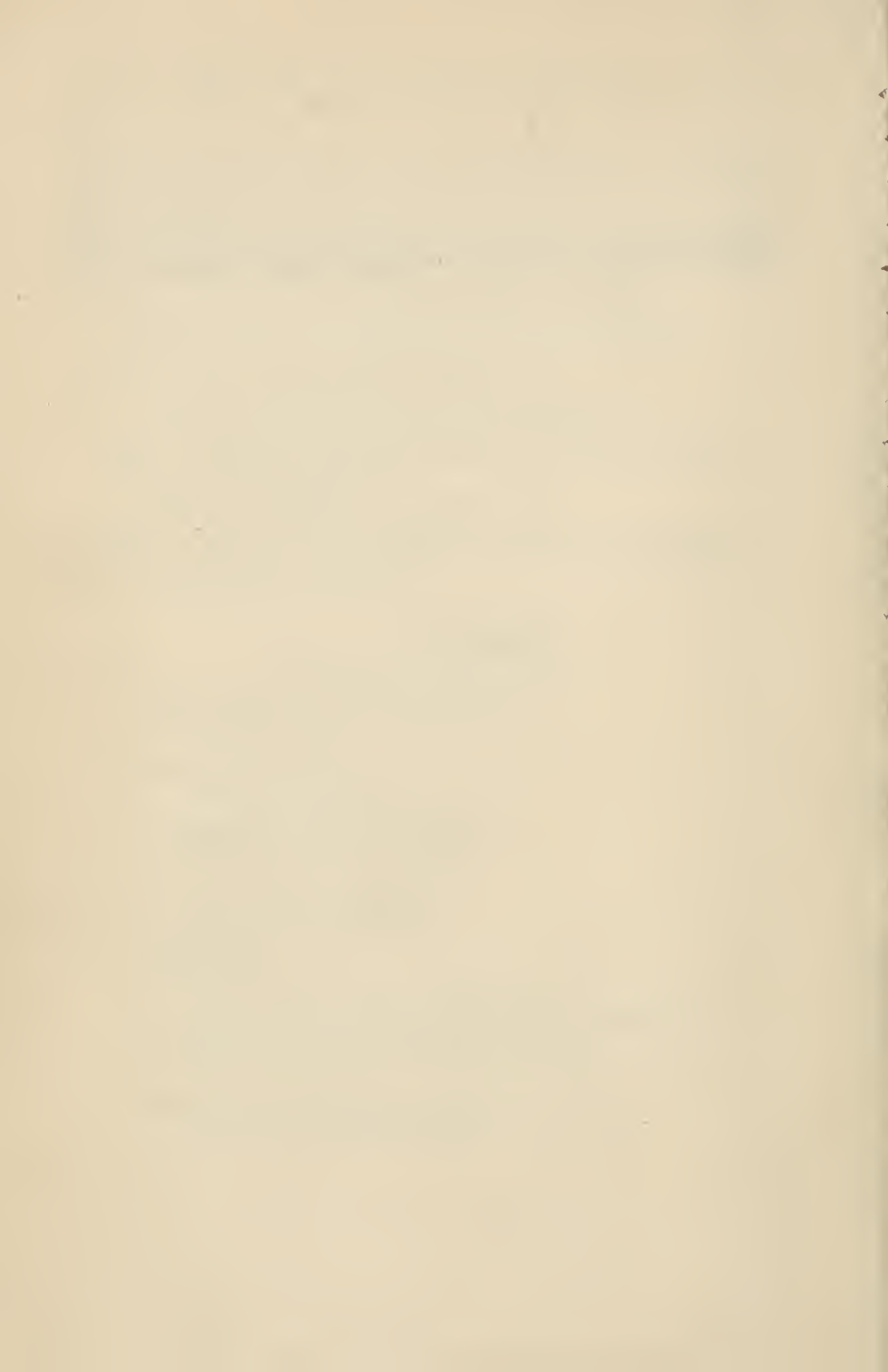APPLIED PHYSICS: ELECTRONICS; OPTICS; METALLURGY
  DIVISIONS 13, 15, 16, 17, 18 AND COMMITTEE ON PROPAGATION OF NDRC

ORGANIZING SCIENTIFIC RESEARCH FOR WAR
  ADMINISTRATIVE FRAMEWORK OF OSRD

ADVANCES IN MILITARY MEDICINE

VOLUME I

Advances in
Military Medicine

MADE BY AMERICAN INVESTIGATORS

WORKING UNDER THE SPONSORSHIP OF

THE COMMITTEE ON MEDICAL RESEARCH

EDITED BY

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VOLUME I

With Illustrations

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PUBLISHER'S NOTE

Under the terms of the contract for the publication of *Advances in Military Medicine,* Volume I, and of the other volumes in the long history of the activities of the Office of Scientific Research and Development, entitled *Science in World War II,* the publisher has agreed to waive its right under the copyright of each separate volume after ten years from the date of publication of such volume. Thereafter the volume in question will be in the public domain and dedicated to the public.

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ILLUSTRATIONS

stop for non-screen x-raying of the small parts, such as the hand and wrist.

In this model of the phototimer the disk is rotated by hand, but pilot lights on the control chassis indicate which stop is in position above the detector, and the symbols on the bakelite plate below the pilot lights remind the technician of the nature of the stop that is being employed. (See Fig. 5.)

a decrease in blood pressure at head level, an increase in heart rate, a loss of blood from the ear, a reduction in the amplitude of the pulse in the ear, and a failure of peripheral vision. There follows a period of compensation during the latter half of exposure in which the blood pressure at head level rises, the ear pulse recovers, blood returns to the ear, the heart rate slows, and vision is restored.

VOLUME II

microscopic appearance of bronchial epithelium is rare at this concentration of diphosgene but is typical at the high ones.)

E: Same conditions as A, but animal was sacrificed at 48 hours. Note lack of dilatation, lack of perivascular edema, columnar epithelium standing in folds, many young epithelial cells, and no marked peribronchiolar infiltration by inflammatory cells. $\times$ 225.

F: Same conditions as E, but no HMT prophylaxis. Note dilatation of terminal bronchiole, necrosis and sloughing of bronchiolar epithelium, little or no epithelial regeneration, accumulation of peribronchiolar inflammatory cells, and edema of perivascular connective tissue. $\times$ 225.

(Navy issue). The left arm was untreated. The temperature in the room at the time of the experiment was 74° F. — R.H. 43 per cent.

FOREWORD

IN this and the following volume are recounted the results of a great co-operative effort in medical research, conducted by the Office of Scientific Research and Development (OSRD) in the interests of our national defense. Written insofar as practicable in nontechnical language, it constitutes a report to the public of advances in medicine which, although primarily designed to promote the health and welfare of our armed forces in camp or in the field, cannot fail to accrue to the permanent advantage of the civilian population. The report is necessarily incomplete and lacking in technical detail; the definitive records will be found in the medical and scientific journals and in scientific monographs. Already more than thirteen hundred papers describing specific aspects of the work have been published, and hundreds more will appear in months to come.

It is perhaps to be regretted that it has not been deemed possible in these chapters to assign credit in the text to all individuals for their contributions, but this omission is consistent with the fact that our country was in danger and with the unselfishness with which physicians and scientists were ardently eager to be used in our national effort.

An account of the organization and administration of the Committee on Medical Research (CMR) can be found in the volume on the administrative history of the OSRD. It has seemed, however, not only appropriate but essential for clear understanding of the circumstances attending its efforts to include in this preface a brief description of the origins of the Committee, its association with the National Research Council (NRC) and other agencies, and the pattern of its administrative management.

The CMR was established by executive order of President Roosevelt on June 28, 1941. The same order created the OSRD as the parent organization within which the previously established National Defense Research Committee (NDRC) and the new CMR were to be constituent agencies. Dr. Vannevar Bush was relieved of the chairmanship of the NDRC and became Director of the OSRD; he thus assumed final responsibility for the entire program of civilian scientific research and development, not only in the fields of instrumentalities of warfare but also in all fields of military medicine.

The CMR was instructed to "advise and assist the Director in the performance of his medical research duties with special reference to the mobilization of medical and scientific personnel of the Nation, . . . to

recommend to the Director the need for and character of contracts to be entered into with universities, hospitals and other agencies conducting medical research activities for research and development in the field of the medical sciences, . . . [and to] submit recommendations with respect to the adequacy, progress and results of research on medical problems related to the national defense."

The executive order specified that the CMR was to consist of a chairman and three members to be appointed by the President, and three other members to be designated respectively by the Secretaries of War and Navy and the Administrator of the Federal Security Agency. The members served without compensation, and with one exception — the appointee of the Federal Security Administrator — the personnel of the Committee has remained unchanged throughout its life.

The more important circumstances that led to the decision that a governmental medical research committee should be established were these: realization of the immensity of the efforts that would be required should we be drawn into the war and of the part that science and technology would inevitably play in the winning of it; demonstration by the NDRC during the twelve preceding months of the competence with which civilian scientists, even though scattered throughout our universities, research institutes, and industrial organizations, could attack problems of warfare when organized under Federal auspices; and recognition of a host of military medical problems in which the Surgeons General needed expert advisory and investigative civilian help but lacked a legally constituted agency having the authority and resources necessary to provide that help.

The first task of the CMR was to become familiar with the problems in which research was needed. In this task timely and vital assistance was obtained from the Division of Medical Sciences of the NRC. In May 1940, the Surgeon General of the Army had asked the Chairman of that division for advice on questions in the fields of chemotherapy and of transfusions. In response to this request two committees of civilian specialists in these fields were appointed. Their usefulness became at once apparent, and similar requests multiplied until in June 1941 there were eight major committees and thirty-three subcommittees with a total membership of 221. By 1943 these numbers had been increased to fifty-two groups with a total membership of 297. Liaison officers from the armed services participated in the meetings of these committees, and it was there that problems were identified and analyzed and the necessity of large-scale programs of research became obvious.

How to initiate, finance, and administer such programs became an urgent question. The National Research Council is not an agency of the government and was not, therefore, in a position to receive direct appropriations from Congress. The funds at its disposal, obtained as grants from the

Federal Security Administrator and from private sources, amounted to only some $70,000 — a sum wholly inadequate for the support of the projects envisaged. This was an impasse that helped to dictate the creation of the CMR as an agency of the OSRD, legally empowered to make effective recommendations for the expenditure of OSRD funds in the fields of military medicine.

The President's executive order contained no suggestions as to the sources from which the CMR should seek advisory aid. But at its first meeting on July 31, 1941, the Committee, recognizing the competence and accumulated experience of the NRC committees, decided to lean heavily on their advice. The Chairman of the Division of Medical Sciences of the NRC was elected vice-chairman of the CMR; the chairmen of the eight major medical committees were appointed consultants to the CMR; and a contract between the OSRD and the National Academy of Sciences was recommended to the Director of the OSRD in amount sufficient to pay the expense incident to the meetings of the NRC committees and to the preparation and distribution of their reports. These arrangements saved invaluable time in the initiation of the work of the CMR. The discussions and decisions of the NRC committees were crystallized into "proposals for research" and recommended to the CMR, with varying degrees of approval. Through the participation of CMR members in the meetings of the NRC committees, through their study of reports, and through their own assessment of the investigators' competence, the CMR arrived at judgments on the basis of which recommendations for investigative contracts with universities, hospitals, etc. were forwarded to the Director of the OSRD. These arrangements continued throughout the existence of the CMR, and, at least until its reorganization in 1944, the major assistance that the Committee received in the initiation of its programs came from the NRC. Too much credit cannot be given to the members of NRC committees who, serving without compensation, interrupted their civilian duties to undertake serious study of the medical problems of the services and to attend frequent meetings in Washington, in order to be able to give competent advice not only to the CMR but to the services and to other government agencies as well.

An idea of the volume of the help that the CMR thus received can be obtained from the fact that a total of 951 proposals for research were considered by the NRC committees, of which 638 were approved and were transmitted to the CMR with appropriate advice. Of these, 501 were recommended by the CMR to the Director and became the basis of contracts for investigative work. In addition to these, 90 proposals were recommended and approved by the Director without prior consideration by NRC committees. The contracts thus entered into involved 135 universities, hospitals, research institutes, and industrial firms. The sums expended amounted to

approximately $25,000,000. The fields of research are shown by the contents of these volumes and by the lists of publications and subjects of contracts.

In their tasks of supervision and co-ordination of research, the members of the CMR were assisted not only by the NRC committees but also by special consultants appointed to make surveys of certain fields. As the number of contract investigations increased to the hundreds, however, the increased burden of administration, supervision, and co-ordination became so great as to require more time and effort than voluntary assistants could justifiably be asked to give. It became impossible for the members of the CMR to maintain familiarity with the multitude of investigations in progress to the extent that the obligations of their appointment required. Hence, during the summer of 1944 at the instance of the Director the organization of the CMR was expanded and given greater formalization. A medical administrative officer was appointed, responsible to the Chairman of the CMR. The broad field of medical research was divided into six divisions: Medicine, Surgery, Physiology, Chemistry, Aviation Medicine, and Malaria. For each division a chief was appointed with formal responsibility through the medical administrative officer to the CMR; each chief had the privilege of appointing section chiefs and technical aides.

In addition to these divisions, a Records Section was established with the duty of receiving the bimonthly, interim, and final reports from the investigators and distributing the information, not only among CMR investigators but also to the Services and to our allies. The Chief of the Records Section inaugurated a weekly bulletin containing abstracts of all unclassified reports, which, in editions that reached 3100 copies, was sent to civilian and military personnel in this country, England, Canada, and Australia and in the theaters of war. This service, which should have been established earlier, did much to fill an important gap in the distribution and hence the usefulness of scientific advances.

As a result of this reorganization, the Chairman and civilian members of the CMR were freed from a mass of detailed work and had more time to devote to formulation of policy. The division chiefs availed themselves of advice from the NRC committees, but at the same time they were authorized to develop new phases of the program. Through their visits and those of the section chiefs and technical aides to laboratories and hospitals, and through conferences with investigators and frequent reports to the CMR, the responsibilities of the Committee were more adequately discharged and the work of many investigators was more effectively supported.

Close liaison between the Services and the CMR was facilitated by the fact that certain of the CMR members and division chiefs also held official positions with the Army or Navy and were therefore intimately aware of the problems of the Services. The Army member of the CMR was the

Chief of the Division of Preventive Medicine in the Office of the Surgeon General, and as such was responsible for research in connection with the health of the troops in camp or in the field; the Navy member was Chief of the Research Division of the Bureau of Medicine and Surgery. One of the civilian members of the CMR served also as a member of the Army Epidemiological Board, created for the investigation and control of influenza and other epidemic diseases in the Army. The CMR Division Chief in Aviation Medicine served also as Co-ordinator of Research in the Office of the Air Surgeon; another division chief rendered important service as Consultant to the Medical Division of the Chemical Warfare Service; a third, as adviser to the Research and Development Branch of the Office of the Quartermaster General, was intimately familiar with all the medical problems of that department.

Many instances could be cited in which CMR investigations became actual collaborations between the CMR and the Services. The following are examples: study of minimal tuberculosis was conducted by a CMR investigator in two Army hospital tuberculosis centers; research on problems imposed by streptococcal diseases became a combined effort by the Army, Navy, a private foundation, and the CMR; the effectiveness of penicillin in syphilis was studied conjointly in Army, Navy, and United States Public Health Service treatment centers and in more than twenty-five civilian institutions under contract with the OSRD; in CMR investigations on fatigue and on water requirements for desert troops, Army troops served as subjects. Throughout the pages that follow many more evidences of co-ordination of effort will be discovered; indeed, the language of the executive order that created the OSRD and the CMR was such as to make obligatory the co-ordination of CMR investigations with those of Army and Navy and other government agencies and the avoidance of duplication of effort.

From the outset the CMR recognized the advantages that would flow from close medical liaison between this country and England. Even before the inception of the CMR, the NRC had arranged with British and Canadian groups for exchange of minutes of their committee meetings. At the first meeting of the CMR, decision was made to establish a liaison office in London, and Sir Henry Dale, President of the Royal Society and member of the Scientific Committee advisory to the British Cabinet, was invited to visit the United States for conferences with the CMR. He came in January 1942, accompanied unofficially by Professor E. D. Adrian. They described the organization and direction of British research and gave helpful advice in the formulation of the CMR program. They agreed that it would be desirable to assign a CMR liaison officer to London, and in February 1942 the first incumbent was installed in the London OSRD office. The medical liaison office was maintained by three successive appointees until

June 1945. These officers attended the meetings of the committees of the Medical Research Council, the Royal Army Medical Corps, and the Flying Personnel Research Committee; they visited research laboratories, and cultivated friendships not only with administrators and investigators but also with high officials in the Medical Services of both the British and the American fighting forces. The information and impressions that they collected were transmitted to the CMR as formal reports and as informal weekly news letters. The CMR in turn distributed to British colleagues the reports of American investigators and the weekly bulletin. In similar fashion, liaison for the British was maintained by a succession of three appointees assigned to Washington. While their primary service was rendered to their compatriots at home, they were also exceedingly useful to the CMR in that they amplified and interpreted the reports of British work and as a result of their assiduous visits to American laboratories were able to give to the CMR competent outsiders' views of the work that our own investigators were conducting.

The proximity of this country to Canada made effective liaison with the latter easy. Representatives of the National Research Council of Canada were frequent attendants at the meetings of the NRC committees and the conferences of CMR investigators; likewise, CMR and NRC representatives were frequently invited to the corresponding sessions in Canada.

These liaison arrangements, effective as they were, could not provide the help that comes from personal exchange of information and opinion between investigators active in the same field. Realizing this, the CMR arranged to send missions of one or more persons to England and beyond and to the theaters of war. In all, some twenty such missions were sent. The principal subjects on which information was collected and the countries or theaters visited, chronologically arranged, are as follows: wounds and burns and their treatment, Pearl Harbor, immediately after the Japanese attack; fatigue and strain of combat aviators, England; industrial medicine problems and armored vehicles, England; chemical-warfare agents, England; neurosurgery, England, Sicily, Italy; venereal diseases, England; penicillin, England; wounds and burns, England, North Africa; aviation physiology, England, North Africa; malaria research, England; psychiatric casualties, England, France, Belgium, Germany; general military medical research, Russia; malaria field research, Mexico, Central America, Peru; malaria, scrub typhus, and field research, Southwest Pacific Theater; tropical skin diseases, the Philippines, Australia, New Guinea; tropical clothing, India, Burma, Ceylon; chancroid, the Philippines. One other mission sent early in 1945 to the Southwest Pacific and the Pacific theaters had general medical survey as its assignment; its inquiry into the distribution of reports of medical advances at home among medical installations in the field revealed distressing inadequacies of transmission of information. For

this reason, among others, plans were made to establish a medical field office in the Philippines through which information might flow to and from the Pacific Theater. The war ended before this plan was put into effect.

Following the success of the Normandy invasion, the Director of the OSRD began to make plans for the orderly termination of its activities. From the beginning of 1945 the CMR was not encouraged to undertake any new commitments other than those of short range and of practical usefulness. As the fall of Germany became increasingly imminent it was decided that all CMR investigative contracts, except those in which a postwar National Science Foundation, should the Congress decide to create it, might conceivably be interested in continuing, should be terminated not later than December 31, 1945. The Services were given the opportunity of taking over under their own contracts such investigations as were of special interest to them; about one hundred such have been transferred. No contract research work continued later than June 30, 1946.

In the foregoing pages allusion has been repeatedly made to "contracts" and "contract investigations." The term is taken from the executive order that created the CMR, and implies more rigid formality in the obligation that a contractor institution assumed than is implied in the term "grant" as used by the NRC, various foundations, etc. For every investigation, a contract was written between the OSRD and the institution in which the work was to be done, containing detailed specifications concerning the amount of the contract, the subject work, obligations concerning progress and final reports, security regulations, cancellation privileges, and the like.

The principle on which the financial terms of the contracts with institutions were based was that of "no loss, no gain." The costs of the research (salaries, equipment, and supplies) were advanced by the institutions, which were reimbursed on presentation of vouchers. Additional sums, called "overhead," calculated as a proportion of the salary budgets, were paid to cover hidden costs of institutional service not susceptible of accurate estimate. During the first two years of the CMR's work, salaries of principal investigators were borne by the institutions; thereafter the contracts were charged for such proportion of their time as was spent on the research.

Although in most cases financial loss to the nonprofit institutions was avoided, there can be no question of the strain put on many of the investigators themselves. By far the greater part of the research work was done in the laboratories of universities, medical schools, and teaching hospitals by members of their teaching staffs. These workers were obliged at the same time to carry on their teaching duties, not at the usual peacetime tempo but at the accelerated tempo of twelve-months-a-year courses. The unfortunate decisions that premedical training be curtailed, that the medical

course be completed in three instead of four years, and that internships be limited to nine months placed demands on the investigator-teachers that would have been more happily borne could they have been convinced of the wisdom of the changes. As a result, time, energy, and freshness for the research were lessened and a large number of our younger physicians have received medical training inferior to the prewar training, which supplied our armed forces in World War II with medical corps of an excellence never before attained.

The executive order contained a stipulation that the OSRD should "utilize the laboratories, equipment and services of governmental agencies and institutions to the extent that such facilities are available for such [research] purposes." The help thus made available, notably through the facilities of the Public Health Service and the Department of Agriculture, was utilized in some of the most fruitful of our investigations; *viz.*, insecticides, antimalarials, and penicillin. Because of the significance of the advances made in these three fields and because their administrative arrangements differed from those of the great majority of CMR contracts, a more extended account appears not out of place in this preface.

INSECTICIDES

The Division of Preventive Medicine in the Surgeon General's Office recognized *ab initio* the menace that insect vectors of disease would present to our troops in foreign combat theaters. At the first meeting of the CMR the service members presented the compelling need for investigations in the field of insect repellents and insecticides. Conferences ensued with officials of the Bureau of Entomology and Plant Quarantine of the Department of Agriculture, which resulted in transfers of OSRD funds to the Bureau with which to amplify its staff and to implement its work in this field. Development of delousing technics, of "lousicides" to be applied to clothing, and of mosquito repellents, far more effective than any before available, was promptly achieved.

As soon as information concerning DDT was received in this country and its potential importance was recognized, the resources of the Bureau were still further increased to enable exhaustive studies of its utility, dangers, and most effective modes of distribution. Inasmuch as that work and its results are described in Volume II and have also been widely publicized through the lay press, description in this preface would be superfluous. Suffice it to mention here the dramatic effectiveness with which the Army aborted the typhus epidemic in Naples in 1943 with DDT and the present confident belief that in this substance an extraordinary agent of sanitation against insect-borne diseases has been discovered.

ANTIMALARIALS

Perhaps the most important medical problem with which the CMR was confronted at its inception was that of malaria, an enemy more to be feared than the Japanese. Deprivation of supplies of quinine by Japanese conquests necessitated search for substitutes. Two possible substitutes had already been discovered by German chemists and pharmacologists — plasmochin and atabrine. The former was quickly judged to be too toxic for such general and indiscriminate use as Army medicine necessitates; atabrine became the drug of choice. In the earlier period of its usage by our medical officers, disconcerting side-effects were reported — gastric symptoms, liver disturbance, lessened mental competence — to an extent that interfered seriously with the enforcement of antimalarial discipline. The first essential was to eliminate the possibility that these effects were due to impurities in the American-made product. Chemical studies, guided by the Chairman of the Division of Chemistry of the NRC, established the purity of the atabrine made in this country. Then, on the advice and with the guidance of the Subcommittee on Tropical Medicine of the NRC, the CMR effected contracts for a fundamental study of the pharmacology of atabrine. Methods were devised for its quantitative estimation in blood, tissues, and urine and applied to the study of human subjects, to whom atabrine was given in various dosages by various routes. The investigators learned how best to attain and how to maintain a concentration of the drug in the blood adequate for suppression of malarial attacks with a minimum of untoward effects. This knowledge became the basis of an atabrine discipline, adopted both by the United States and by the British Medical Corps, the enforcement of which reduced malaria from its position as a menace more dangerous than Japanese bullets to one of relative inconsequence.

Simultaneously with the atabrine studies, an organized search was initiated under the CMR for hitherto undiscovered antimalarials under the leadership of the Chairman of the Division of Chemistry of the NRC. The shelves of academic laboratories of organic chemistry and of the research laboratories of chemical and pharmaceutical manufacturing firms were scanned for substances of theoretical promise. Academic chemists were encouraged to invent new synthetics for experimental trial. A survey office was established in which a record was kept of the constitution of every substance that became available for trial, of its toxicity in animals, and of its effectiveness against avian malaria. (Eventually more than fourteen thousand compounds were thus screened.)

It soon became apparent that facilities would be required for the experimental testing of new antimalarials on volunteers infected with malaria, and that the relatively simple arrangements made by the Division of

Chemistry and the Subcommittee on Tropical Diseases were inadequate for a task of such magnitude. A special Committee for the Co-ordination of Malaria Studies within the NRC was therefore appointed by the Chairman of the Division of Medical Sciences, with subsidiary panels on synthesis, biochemistry, pharmacology, and clinical testing. Subsequent progress and prospects of success, together with recognition of need for closer co-operation with the malarial work of the Services, then led to the creation of a board independent of the NRC, of the OSRD(CMR), and of the Services but composed of appointees from each. Under recommendations from this board and its constituent panels, the CMR program was expanded and the work accelerated, particularly with respect to the synthesis of new compounds and their trial in volunteers. These volunteers were found among conscientious objectors relieved of military service, among patients in civilian hospitals, and among inmates of federal, state, and military prisons. The patriotic zeal with which the last-named group submitted themselves to infection with *vivax* malaria, with its distressing periodic recurrences, is particularly laudable. The official record of these malaria studies has been assembled in three volumes brought together by the Board and published by the OSRD. Numbers of new compounds having greater promise as effective antimalarials than either quinine or quinacrine were disclosed. Two of these, one a highly effective suppressive, the other having definitely curative power against *vivax* malaria, have been subjected to field trials and give promise of the beginning of a new era in the field of antimalarial medication.

PENICILLIN

The wartime advance made in this country in the development of penicillin as an available therapeutic agent was the result of a unique co-operation among government agencies, civilian investigators, and pharmaceutical manufacturers. Supreme credit for penicillin belongs, of course, to Fleming of London, the discoverer, and to Florey, Chain, and their colleagues of Oxford, the first demonstrators of penicillin's possible effectiveness. But the great task of transforming a flask and test-tube experiment into an industry capable of furnishing investigators a sufficient supply for convincing trials, and armies enough for their manifold needs, was given to American enterprise to accomplish.

Professor (now Sir Howard) Florey came to this country in July 1941. Animal experiments and several trials of its therapeutic effectiveness had convinced him that penicillin, could it be made available, would prove to have great importance in war medicine. He hoped to stimulate interest among mycologists and pharmaceutical manufacturers here that would result in the production of enough penicillin to enable extensive studies of

its therapeutic potency and limitations and ultimately enough for distribution to the Services. British science and industry were so engrossed with war projects of more thoroughly demonstrated necessity as to make the initiation of such an effort in England impossibly difficult.

Florey was referred by the Chairman of the NRC to the mycologists of the United States Department of Agriculture at the Northern Regional Research Laboratory at Peoria, Illinois, and they, greatly interested, began at once a study of the characteristics of Florey's cultures of penicillium. Florey's conversations with the Chairman and other members of the CMR led them to share his convictions and to decide to attempt the promotion of the effort he so ardently desired. In October 1941, a meeting was held in Dr. Bush's office to discuss the possibility of a co-operative effort to produce penicillin in quantity. Present were Dr. Bush, the Chairman and Vice-Chairman of the CMR, the Chief Mycologist of the Department of Agriculture, the Chairman of the Division of Chemistry of the NRC, and the scientific directors of four large Eastern pharmaceutical manufacturing houses. A similar meeting was held in December 1941, at which the heads of the same manufacturing companies and the Chief of the Fermentation Division of the Northern Laboratory were also present.

From these meetings, all the participants received assurance of the interest of OSRD(CMR) and of the support that the OSRD was prepared to supply to their efforts. It was specifically agreed that the Peoria laboratory would energetically continue its study of the cultural characteristics of the mold with a view to increasing its productiveness; that the Chief of the Fermentation Division would advise the co-operating companies of progress made and act as their consultant; and that, while the companies would proceed with production research independently, their progress would be reported to the CMR for such distribution as would advance the program.

Early in 1942 the OSRD made funds available to the Northern Laboratory for extensive expansion of its work. The industrial companies chose not to apply for government funds; indeed, for more than a year after these meetings the only penicillin that the CMR distributed for clinical studies was provided by three of these companies without cost to the government. Priorities for the apparatus and equipment necessary for production research by the industrial firms were obtained from the War Production Board through the advice of the CMR. Unfortunately it was not within the power of government to grant priorities that could mitigate the strain and struggle endured by the production engineers of the pioneering companies, faced with the problem of growing a sensitive and fastidious mold in thousands-of-gallon quantities and extracting in pure form from those cultures an unstable substance present in concentrations of only a few hundredths of 1 per cent.

In December 1941, at the instance of the CMR, the NRC Committee on

Chemotherapeutic and Other Agents was requested to initiate and co-ordinate an extensive study of the therapeutic uses and limitations of penicillin. This study was carried on with extraordinary competence in selected clinics, and by March of 1943 the results of treatment of 200 cases with penicillin were available. They were so impressive that in April of that year, by arrangement with the Surgeon General of the Army, one of the civilian investigators under the CMR was invited to conduct a study of the effects of penicillin in the treatment of compound fractures and osteomyelitis in wounded soldiers from the Pacific Theater at the Bushnell General Hospital. The success of this study led to the initiation of programs of study and indoctrination in Army hospitals, which were antecedent to the general adoption of penicillin throughout the Army medical services.

Concurrently with these developments, the War Production Board came to recognize the necessity of greatly expanded facilities. They surveyed the country to identify the plants most adaptable to penicillin production, saw to it that equipment was available, and through their Office of Production Research and Development obtained and distributed information that greatly increased production effectiveness. By D-Day (June 6, 1944), thanks to superb co-operation among government agencies, research and production teams in commercial organizations, and clinical investigators in many civilian hospitals, our forces in England were supplied with enough penicillin for the treatment of our own battle casualties from the Normandy invasion as well as those of our British allies. The cost of the whole program that the OSRD was called on to bear was relatively trivial, the major item in it being the expense of the penicillin supplied to the clinical investigators after February 1943.

Another important although less successful co-operative enterprise sponsored by the CMR was directed toward the synthesis of penicillin "or its therapeutic equivalent." From the beginnings of the work of the Oxford group, chemists entertained the expectation that once penicillin had been obtained in pure crystalline state its constitution could be determined and its synthesis effected. Such a contingency provided a serious element of risk to the investments of large sums by the commercial companies in plants designed for production by fermentation. A special committee of chemists was appointed by the Director of the OSRD to survey the situation and to name the groups of chemical investigators best able to attack the problem of constitution and synthesis. Contracts were written with eleven commercial firms, five academic institutions, and two government agencies. Again the commercial firms chose not to accept financial support from government. The contracts provided for full exchange of information among the groups and, should the work give rise to patentable discoveries or inventions, constituted the Director of the OSRD as the final arbiter in the determination of patent rights, royalty payments, etc. among the participating organizations.

At the instance of the Director of the OSRD, a similar organization of chemical effort was inaugurated in England through the efforts of Sir Edward Mellanby, Secretary of the Medical Research Council, with similar provisions for pooling of knowledge and disposition of patent rights. Exchange of information between the two countries was as complete as circumstances would permit, and an agreement between the governments of the United States and the United Kingdom was consummated for equitable settlement of patent claims and filing rights, not only within the two contracting nations but in other countries as well. The purpose for which the Director of the OSRD strove so effectively was the avoidance of a patent-controlled commercial monopoly that might exploit a new drug of unique medical importance. The wholehearted concurrence of the participating firms with this purpose speaks volumes for their patriotic spirit. Although a practicable synthesis of penicillin has not been achieved, much new and valuable information concerning the constitution of penicillin and of its degradation products has been brought to light. Fortunately, because of amazingly effective improvements in methods for large-scale production of penicillin by fermentation, an unlimited supply has become available at very low cost to the public.

In bringing this preface to a close, the greatest emphasis should be laid on the unselfish zeal, co-operative spirit, and competence with which our civilian investigators, laying aside more agreeable pursuits, entered into the attack on problems whose solution was vital to our fighting forces. The conjoint effort thus put forth involved nearly seventeen hundred doctors of medicine, philosophy, or science and thirty-eight hundred scientifically trained associates. Never before, we believe, has there been so great a co-ordination of medical scientific labor. It was conducted under the stress and strain of national peril and urgency. In many instances the work was a heavy addition to already increased civilian tasks. Regimentation, supervision by, and periodic reports to administrative authority, distasteful as they are to pioneers, were borne with tolerant forbearance. Security regulations, although minimized to an extent by the ruling of the Secretaries of War and Navy that only medical information having military value should be kept from publication, were a deterrent to the co-operation for which all were eager. Many of our younger investigators were torn by the question whether their highest duty was not to enter military service, and the compunction with which they applied to Selective Service local boards for deferment cast a cloud of perturbation over their work. We hope that they can all take comfort in the fact that their efforts saved lives, diminished disabilities, and shortened the war. The increase in number and quality of preventive and therapeutic resources that has been achieved will redound to the future health of all nations and hence may compensate, in a measure at least, for the injuries that our forces, in the national defense,

were forced to visit on others. The CMR takes pride in the privilege of having been able to provide this great group of investigators with the means for their work and to help in making it useful to those who fought.

Repeated reference has been made in this statement to the invaluable help received by the CMR from the NRC committees and from other advisers. Without it we should certainly have stumbled and might have fallen.

A special debt of gratitude is owed to those who, when the heat and burden were removed, have accepted the tasks of writing the chapters and sections of these two books. The writing has been done despite fatigue, accumulations of civilian duties long postponed, and necessities of planning for future activities.

Finally, a word of thanksgiving must be uttered for the good fortune that gave to this country a scientist to be chosen as Director of the OSRD whose qualities both as a leader and as a servant could not have been surpassed. His competence in physical science and in administration was long ago proved; to those in the field of medical research who have served under Dr. Bush, his discriminating support, together with his understanding of the human heart, has been a tower of strength.

ALFRED N. RICHARDS
*Chairman, Committee
on Medical Research*

ADVANCES IN MILITARY MEDICINE

VOLUME I

Part One: Medicine

INTRODUCTION

E. COWLES ANDRUS

WAR AUGMENTS certain medical problems already existent in civilian life and engenders new ones. There are factors involved in greatly enlarging an army and navy that pose special difficulties from the medical standpoint. This process brings together in large numbers presumably healthy young persons and subjects them to an especially vigorous type of physical training. On the face of it this should be advantageous to the health of most, and so it is, but there are particular hazards that have soon become evident in past wars and that did so in the recent emergency. The first of these hazards is inherent in the inevitable herding together of persons from different localities who are variously susceptible to infectious diseases. In past wars, epidemics at home and abroad have sometimes been as destructive as battles.

Guided by its experience in World War I, the Army prepared to study and to control communicable disease by appointing the Army Epidemiological Board, with its various constituent commissions of experts. These groups conducted field studies and clinical investigations in service installations. Their results are available in the published medical literature. Collaborating with them in the interest of both Army and Navy were the Committee on Infectious Diseases and the Committee on Medicine of the National Research Council. On the advice of these committees, the Committee on Medical Research sponsored laboratory studies to develop new methods of protection against several important types of infection and organized research designed to improve the treatment of others.

One of the most serious hazards is venereal disease, which constitutes a recurrent problem of armies and navies. The efforts of the Committee on Venereal Disease of the National Research Council and the support of the Committee on Medical Research were early addressed to this problem during the recent emergency. The most spectacular phase of this collaboration was the study of the treatment of venereal disease with penicillin.

Another hazard derives from the necessity of sending members of the armed services into regions of the world where there are prevalent types of disease not encountered at home. This has required that the scope of civilian investigation of tropical diseases be greatly broadened and accelerated. In addition, it became necessary in the recent war to do everything possible to provide protection against two diseases that have been almost completely unknown in the United States since the organization of modern methods of public health and sanitation — cholera and plague. Tropical diseases have been known to practicing physicians of this country only in certain special localities about ports.

In the armed services steps were taken to familiarize the medical personnel with the manifestations of tropical diseases. The recognized danger that members of the armed services would be exposed to these diseases in the tropics stimulated research in civilian institutions under the aegis of the Committee on Medical Research. In this program the Committee again depended on the advice and assistance of the various constituent committees of the National Research Council through its Division of Medical Sciences.

Finally, the improvement of methods of treatment of disease and injury itself engendered new problems in the field of convalescence and rehabilitation. The death rate from battle injury in World War II was the lowest in history, but this did not mean that all survivors could be quickly restored to health. Organized collaborative research in this field had only begun at the end of the emergency.

It should be appreciated that the research efforts described in this section are not reported in any degree of completeness. Various aspects of certain problems were of intimate concern to different fields of medical science, and some studies for which the Division of Medicine was in part responsible are more appropriately described elsewhere in this volume. For example, the studies of clostridial toxoids were correlated with the investigations of gas gangrene made by the Division of Surgery. The metabolic derangements of convalescence are to be considered in their relation to the other fundamental metabolic and nutritional studies described in the section on physiology. The chemotherapeutic observations incidental to special studies of particular infectious diseases are only briefly mentioned. The psychiatric observations of post-traumatic personality are included in this section but cannot be dissociated from the surgeons' reports of head injuries and neurosurgery.

Indeed, a principal advantage of the organization of the Committee on Medical Research was its flexibility and the spirit of co-operation between the various divisions. Centralization of activities as far as possible in one accessible building helped greatly to foster this spirit.

INFECTIOUS DISEASES

ALPHONSE R. DOCHEZ, COLIN M. MACLEOD, AND JAMES E. MCCORMACK

O F THE many triumphs over human disease for which modern medicine deserves credit, perhaps none is more notable than the power to control the activity of the micro-organisms that cause infectious disease. From the time of the discovery by Pasteur of the essential relationship of bacteria to certain diseases of mankind, progress in the direction of prevention and cure has been rapid and continuous. In fact, many of the destructive plagues that in past ages brought death and disability to vast populations of the world have now entirely disappeared or become inconsequential wherever modern medical technics are understood and efficiently applied.

Furthermore, such infectious diseases as are still prevalent are yielding in an almost miraculous manner to the more recent advances of medical science. Medicine finds its most appropriate field of successful activity in the stabilized populations and efficiently organized communities that progressively evolve during periods of peaceful and harmonious human relations. In wartime, on the other hand, the varying disruptive influences of military conflict disturb the favorable effects of this equilibrium and promote the development of adverse situations in which bacterial infection finds renewed and widespread opportunities for dissemination.

Pathogenic micro-organisms gain entrance to the human body by certain well-established routes. In warfare the break in continuity of skin protection, the crushing of tissues, and the interruption of blood supply that accompany wounding are among the leading causes of serious bacterial infection. Other significant portals of entry are the respiratory and gastrointestinal tracts and the skin, through inoculation by insect vectors. The diseases induced in these various ways comprise the wide range of septic conditions and specific fevers, which are common enough during peacetime but that during the periods of warfare, unless adequately controlled, tend to increase to a dangerous extent. In addition, the geographical displacement of large groups of people, incidental to war, results in exposure to unaccustomed types of infection and in certain instances to disease of incompletely understood character and unknown causation.

Thus the medical problems in the field of infection become numerous and varied and demand an elaborate and well-trained medical organization for their effective control. Although the principal burden for the control of infectious diseases during the war was naturally the responsibility of the Surgeons General of the military services, the Office of Scientific Research and Development, at the request of the armed services, participated in the study of a number of specific conditions.

BACILLARY DYSENTERY

In the now somewhat remote past, infections affecting the gastrointestinal tract, such as typhoid fever and the bacillary dysenteries, were a fearsome scourge of armies in the field, causing many deaths and an extraordinarily high rate of ineffectives. Already in World War I the enteric fevers had been brought under adequate military control through improved methods of sanitation and the general application to troops of the protective measure of prophylactic immunization. The bacillary dysenteries, on the other hand, have proved more resistant to control measures. There are a number of reasons for this state of affairs. Effective sanitary protection of water and food supplies available to rapidly moving troops under combat conditions and in areas where the risk of dysenteric infection is great presents at times almost insuperable difficulties; preventive inoculation against dysentery has never reached the stage of practical and successful application; finally, the carrier rate for dysentery bacilli attains an extremely high incidence and the carrier state is frequently maintained for long periods of time. The risk of attack by dysentery is therefore great, both from the general contamination of available water supplies and from the local contamination of food by more or less healthy carriers.

The Office of Scientific Research and Development undertook to aid in the solution of this problem in two different ways. It was decided on the one hand to explore the possibility of preparing a useful vaccine, such as is employed against typhoid fever, and on the other to ascertain the relative curative value, both for the acute disease and for the carrier state, of such sulfonamide drugs as had already proved useful.

From the time of the discovery of the dysentery bacillus by Shiga, frequent attempts have been made to prepare a practical and effective vaccine for use in human beings. These efforts have been on the whole unsuccessful, for a number of reasons. The effectiveness and duration of the immunity that follows a natural attack of dysentery have never been satisfactorily determined. Experience, however, suggests that persons exposed to repeated infection with the dysentery bacillus do after a time acquire a certain amount of resistance to the disease. Furthermore, there exist a multiplicity of specific types of this organism each of which evokes an immunity peculiar to itself,

with no protection afforded against infection with types of different serologic specificity. The prevalence of infection with the different types of dysentery bacillus varies greatly in different geographical areas of the world, so that it was not known whether immunization of mass populations would be of value in prevention, nor was it clear which of the multitude of bacterial types should be included in a stock vaccine. A further difficulty in the use of dysentery vaccine is the inherent toxicity of the organism itself, inoculation with which in adequate amounts may induce such severe local and general reactions as to make general application among troops impractical. Aware of these facts, the Committee on Medical Research assigned various aspects of the dysentery problem for investigation by laboratories throughout the country.

Early experience in the clinical use of the sulfonamide drugs indicated that they would exert a highly beneficial effect on infections caused by the dysentery bacillus. A number of contracts were therefore entered into by the Office of Scientific Research and Development, designed to assemble reliable information concerning how many of the available sulfonamide drugs were useful in the treatment of dysentery and what was their relative efficacy for this purpose. To accomplish this objective it was necessary to study forms of dysentery caused by different types of bacillus, because of their varying susceptibility to the chemotherapeutic action of the drug. The study involved a consideration of dosage, of method of administration, and of treatment, both of patients in the acute phase of the disease and of convalescent and healthy carriers of the dysentery organism.

At the beginning of this investigation information was already available concerning the capacity of certain sulfonamides to check the growth of the dysentery bacillus on cultivation in vitro, and sulfaguanidine had been tested for its efficacy in human infections. The in vitro action of the drugs was confirmed, and four easily available ones — sulfaguanidine, sulfasuxidine, sulfathiazole, and sulfadiazine — were found to hold the greatest promise of usefulness in all the common types of dysentery.

The problem of testing the efficacy of these drugs in sufficiently large numbers of cases of human dysentery at first appeared rather formidable, but through the co-operation of the United States Public Health Service and its trained staff the study was satisfactorily organized. A number of institutions, either hospitals or establishments devoted to custodial care where dysentery was known to be prevalent, were selected for the study. Hospital cases were used to determine the curative value of the sulfonamide drugs in acute cases of the disease. The institutions with a permanent population served for a study of the epidemic spread of dysentery, the relationship of carriers to this situation, and the scope of sulfonamide therapy as a control measure. Abundant acute cases were also available for therapeutic tests.

The varieties of dysentery bacilli most frequently found in association with

epidemic outbreaks of the disease in the United States are, in the order of prevalence, the Flexner, the Sonne, and the Boyd 88 types. Infection with the Shiga bacillus is rare. Acute infections with any one of these organisms can be terminated by the administration of a suitable sulfonamide drug. Of the different varieties of dysentery bacilli the Flexner types are the most sensitive to the action of the drug, the Schmitz bacillus (*Shigella ambigua*) occupies an intermediate position, and the Sonne type is the most resistant, different strains of the last-named organism showing considerable variation in their sensitivity to drugs. All types of infection being considered, three sulfonamides — sulfadiazine, sulfapyrazine, and sulfasuxidine — have superior records. Sulfamerazine and sulfamethazine are a little less effective, although the former appears to be highly active against infection from the Flexner types. Sulfathiazole and sulfaguanidine proved the least satisfactory of the seven sulfonamides that were widely tested. Considering availability, toxicity, and relative efficacy, sulfadiazine is thought to be the drug of choice in the treatment of bacillary dysentery. However, in certain examples of the Sonne type sulfasuxidine has proved to be of special value.

Notwithstanding the discouraging experience in the past with dysentery vaccine as a preventive of this disease, at the suggestion of the Army renewed attempts toward its improvement were stimulated. These efforts were particularly oriented toward reduction of the toxicity of the vaccine. A study was first undertaken to ascertain the degree of resistance that follows an attack of dysentery. Men who have moved from nonendemic areas of dysentery into endemic areas frequently report one or two attacks of clinical dysentery during the first year of sojourn in the endemic region; thereafter they are relatively resistant. A survey of hospital records was conducted at the Harriet Lane Home in Baltimore, where a number of children suffering from bacillary dysentery are treated each year, in order to discover whether the impression concerning acquired resistance could be confirmed. The subsequent incidence of dysentery in children admitted to the hospital with the disease was compared with that of a similar group admitted because of other conditions. The survey, which embraced an average follow-up period of three and a half years, demonstrated that one attack of bacillary dysentery confers appreciable immunity against subsequent attacks. The existence of naturally acquired immunity strengthened the hope that an effective vaccine might be produced.

Because of the multiplicity of types of dysentery bacillus and because of the varying incidence of disease due to different types in the many areas of military activity, consideration was given to choosing the most suitable strains for incorporation into a vaccine. Determination of the types prevailing in current outbreaks of dysentery was proceeding in laboratories of the Army, Navy, and United States Public Health Service and elsewhere, so that it was possible for the various investigators to agree on five or six types,

immunization against which, if practicable, should suffice in the great majority of outbreaks.

As part of this study, early investigations were directed toward discovering individual dysentery strains possessing broad cross-immunizing characteristics against all types of Shigella bacillus. This search was not entirely successful. It was repeatedly demonstrated that vaccines prepared from each of the better known V, W, and Z Flexner types are able to evoke significant agglutinins and mouse-protective antibodies against the other two heterologous Flexner types, but the homologous immune response is always greater than the heterologous response. As regards the foregoing Flexner types and the Boyd 88 type, there are slight and immunologically unimportant cross-immunizing characteristics. No cross-immunizing activity whatever has been demonstrated between *Shigella paradysenteriae, Shig. sonnei,* and *Shig. ambigua.* This knowledge helped to define strains that would have to be incorporated in a polyvalent vaccine.

Dysentery vaccines when administered subcutaneously in man in high dosage are capable of evoking severe untoward manifestations. The systemic reactions include fever, malaise, nausea, vomiting, diarrhea, cyanosis, and injection of the conjunctivas; such symptoms rarely persist for longer than twenty-four or thirty-six hours. The local reaction includes erythema, induration, swelling, and tenderness about the site of injection and is usually associated with tenderness and swelling of the regional lymph nodes. At lower dosage levels systemic reactions are largely eliminated and the local reaction is mild. In general, the immune response, as measured in mouse-protection tests, tends to parallel the severity of the reaction. Moreover, the margin of safety — between dosages that are tolerated without discomfort but evoke an unsatisfactory immune response and those that cause severe reactions and an elevated immune response — is not wide; it is of the order of twofold or threefold.

These circumstances have compelled careful evaluation of the maximum tolerable dosage in human beings. The criterion has been that an acceptable dysentery vaccine shall not evoke a severer reaction than is elicited by the commonly used typhoid vaccines. The present consensus favors a vaccine containing roughly 2400 million bacterial cells per cubic centimeter. Such a vaccine is intended for use in 0.5-cc., 1.0-cc., and 1.5-cc. doses, administered subcutaneously at weekly intervals. A number of pentavalent vaccines containing dysentery types Flexner V, W, and Z, Boyd 88, and Sonnei have been evaluated clinically at or near this dosage level. Reactions have not surpassed the limits of safety, and the immune responses have been sufficient to give some promise of effectiveness.

In several laboratories work under contract with the Office of Scientific Research and Development has been concerned with attempts to diminish the toxicity of the dysentery vaccine without parallel loss of immunizing

capacity. Dysentery bacilli contain a substance that endows the bacterial cell with its toxic and specific immunizing properties. This specific cell-body substance comprises about 10 per cent by weight of the bacterial body and can be extracted in its entirety with certain organic solvents. Chemical purification yields a complex molecule consisting of a phospholipid, a protein, and a carbohydrate. These substances in combination are highly toxic. When injected into animals and man they give rise to immune bodies that both agglutinate the homologous organism and protect mice against infection with lethal doses of the appropriate dysentery bacillus. The immune bodies elicited have proved to be indistinguishable from those evoked by injection of the intact, killed bacilli themselves.

Attempts have been made to detoxify these agents so that they may be used for purposes of preventive inoculation. Chemical and physical technics of a wide variety have been employed for this purpose, but most of these manipulations have proved to be more destructive to the immunizing moiety than to the toxic component. It was found that when the pure somatic antigen of the Z Flexner type of dysentery bacillus was treated with periodic acid, both the immunizing power and the toxic effect were rapidly destroyed. When type Z bacilli were heat-killed and then treated in a similar manner, however, the micro-organisms were rapidly rendered nontoxic, yet their ability to incite antibacterial immune bodies in experimental animals was not greatly impaired. The successful application of this method has been extended to another member of the Flexner group of dysentery bacilli.

A second method for detoxifying the dysentery antigen while preserving its immunizing value consisted in the employment of ultraviolet light or heat in the presence of ozone or hydrogen peroxide. With this method damage to the protective power of the antigen was markedly lessened by performing the detoxification in the presence of sodium caprylate and gum arabic. This represents the first hopeful result in the many attempts to detoxify dysentery bacilli without at the same time destroying their immunizing value. The preparation of vaccines for human administration detoxified by this procedure awaits future study and evaluation.

Related attempts have been made to diminish the toxic effect and enhance the immune response by preparing vaccines in which the bacterial cells are suspended in an oil-emulsion base. Subcutaneous injection of these vaccines elicits a transient acute inflammatory reaction and a persistent granulomatous nodule. Experiments in mice have shown persistence of antigen in the granuloma for as long as sixteen weeks. The height and duration of the immune reaction are markedly increased by this method in comparison with the effect attained by use of an equivalent vaccine suspended in saline solution. Further studies are necessary in order to define the intensity and duration of the immune response in human beings as well as the practicability of the procedure.

An interesting development has been the demonstration that by constructing acetylated derivatives of the dysentery bacillus antigen the toxicity can be reduced to one sixtieth or less of the toxicity of the native substance. Vaccination of mice with these materials protects them against fatal doses of living dysentery bacilli.

Vaccines have been prepared for use in the field in accordance with the information derived from these studies, but to date these vaccines have not been tested on military personnel. In certain civilian institutions where outbreaks of dysentery are not uncommon, opportunities have been furnished to observe the effect of the vaccines under approximately field conditions. The vaccines used were polyvalent ones containing the strains of dysentery bacillus previously mentioned; in one instance a monovalent vaccine was directed against the prevalent epidemic strain. The vaccines were inactivated either by formalin treatment or by ultraviolet irradiation. Vaccination failed either to reduce the number of cases or to lessen their severity. The course of one well-studied outbreak was modified little if at all by vaccination.

The evidence to date does not encourage the hope that dysentery vaccines as prepared and given at present will have any practical value in the control of dysentery. Whether these results will be improved by the use of vaccines detoxified by more recent methods so that larger doses can be administered remains to be seen.

Although bacillary dysentery due to the Shiga type of bacillus is uncommon in the continental United States and in Europe, it was foreseen that this organism might become an important cause of dysentery in case of large-scale land operations in China or Japan. A special study was therefore inaugurated, which was limited to study of infections caused by the Shiga bacillus.

Shiga bacilli differ from the other members of the dysentery group in giving rise to cultures that, when grown under certain experimental conditions, exhibit a high degree of toxicity for experimental animals. It is true that cultures of virulent forms of other dysentery bacilli (the Flexner, Boyd 88, and Sonne strains) also contain toxic materials, but in the case of these strains the whole toxic activity resides in the specific somatic antigen and is of a lower order than that described for the Shiga group. The high toxicity of the Shiga cultures is due to the production of another toxin, which, because it is released in solution in the medium and because it gives rise to paralytic manifestations in experimental animals, has been designated as the Shiga exoneurotoxin. Since Shiga infections in man are often characterized by a high degree of toxemia, it is probable that this exotoxin plays a part in determining the severity of the disease, and it therefore appeared possible that the production of antitoxic immunity might be a desirable goal. Consequently, efforts were directed toward the development of rapid and dependable methods for the production, concentration, and purification of

Shiga exotoxin, and for its conversion into a nontoxic antigenic product (toxoid) that could be used for immunization.

It had long been known that the Shiga exotoxin is produced chiefly when the culture is incubated under aerobic conditions. It was established that the yield of bacterial protoplasm synthesized and of active toxin produced in liquid media is greatly increased by causing forced aeration of the culture throughout the course of incubation; this can be achieved by violent agitation or by passage of a stream of fine air bubbles, as is the practice in the production of penicillin. Under these conditions of forced aeration, complete oxidation of metabolites takes place and, for example, glucose can be added to the medium without accumulation of organic acids and lowering of the pH. Aerobic metabolism is also favored by addition of 0.1 per cent fumaric acid to the medium. The effect of this substance is reflected not in an increased yield of bacterial cells but chiefly in a marked increase in the toxicity of the bacterial protoplasm produced.

Earlier investigators had also recognized that better yields of toxin are obtained in alkaline media — at pH 8.2, for example. It is known that inorganic iron becomes insoluble at this pH (owing to the formation of insoluble hydroxides and phosphates); on the other hand, recent work has established that the diphtheria and tetanus bacilli produce their respective toxins in satisfactory yields only on media deprived of inorganic iron. It was found that in the case of the Shiga dysentery bacillus also, toxin production is greatly increased in media that have been freed of inorganic iron; in fact, it is possible to obtain satisfactory yields of toxin by growing the organism for twenty-four to thirty-six hours at neutral pH in iron-free media.

Toxin production by Shiga bacilli is independent of the virulence of the culture, and an avirulent R Shiga variant has been obtained that is highly toxigenic. For a number of different reasons this particular strain presents advantages for the development of large-scale methods of toxin production, purification, and detoxification. It has been used in preference to the S virulent strains in the practical method that has been elaborated.

The Shiga toxin is rapidly released in solution as the bacterial cells die and begin to undergo autolysis. It can be concentrated and purified by taking advantage of the fact that it is precipitated as an insoluble salt of nucleic acid when the culture filtrate is acidified to pH 4. However, the nucleic acids, which are present in high concentration in the crude preparation of soluble toxin, are not essential to its biologic activity. They can be removed by a number of technics, in particular by precipitation with calcium chloride at alkaline pH, without affecting the toxicity of the preparation.

On the basis of these observations a rapid and dependable method of toxin production has been developed that involves the following steps:

Avirulent R Shiga bacilli are grown under conditions of extreme aerobiosis in a simple medium at pH 7 containing inorganic salts, peptone, meat

extract, glucose, and fumaric acid. Maximum toxin production is obtained within twenty-four to thirty-six hours. The toxic principle is separated from the cellular material by filtration through filter paper at alkaline pH and precipitated by acidification of the filtrate to pH 4. Treatment of concentrated solutions of toxin with calcium chloride at pH 9 gives an inert precipitate containing 50 per cent of the total nitrogen and 95 per cent of the total phosphorus, whereas the filtrate retains the toxic activity of the original material. The most active preparations of toxin available exhibit an LD_{50}[1] of 1 to 5 μg. for rabbits and mice; in other words, Shiga toxin has been obtained in a form possessing a biologic activity comparable to that of other classical toxins.

The concentrated soluble toxin can be readily detoxified by formol. Detoxification does not take place when the formol treatment is carried out at neutral or acid reactions, whereas treatment at alkaline pH may cause a loss of specific antigenicity at the same time as detoxification takes place. It is very important, therefore, carefully to control the pH of the system during the treatment with formol. In practice, optimal production of toxoid is obtained by adding 0.5 per cent formol to a concentrated solution of toxin (containing 700 to 1000 mouse LD_{50} per milliliter) and incubating the mixture at 37° C. for two or three weeks, the pH being carefully maintained between 8.4 and 8.6 during the whole process. Under these conditions, there takes place a decrease in toxicity of 300 to 500 per cent, whereas the antigenicity is maintained unimpaired. The toxoid can be precipitated with alum, and a single injection of 0.1 ml. of this alum toxoid by the subcutaneous route is sufficient to establish in mice a state of active immunity against the toxin.

Toxoid prepared by similar methods has been injected in man by other workers and found to elicit appreciable antitoxic response without causing untoward reactions. Although no information is available for evaluating the possible effect of immunization with toxoid on the susceptibility of man to Shiga infections, it may be surmised that an adequate level of antitoxic immunity would decrease the severity of the disease by minimizing the profound toxemia usually associated with it.

ASIATIC CHOLERA

Investigation of Asiatic cholera was promoted by the Committee on Medical Research with a view to discovering to what extent the methods available for the control of this disease could be improved. Cholera was not an urgent operational problem in the Pacific areas, but the disease has long been endemic in India, and widespread and severe outbreaks occur there with considerable frequency. Furthermore, in the seaports of China and the Burma

[1] The amount necessary to cause death in 50 per cent of animals tested.

area cholera is frequently prevalent, and the risk of infection among troops operating in these areas might be great.

Of the practical methods available for protection against cholera, prophylactic vaccination seemed to be the most promising for further evaluation. Before the onset of the war heat-killed cholera vaccines had been extensively used in India and Japan. Although the conditions under which they were employed were not always favorable enough to permit satisfactory statistical analysis, most of the reported results indicated that the vaccines were protective and that they assured sufficient reduction in morbidity and mortality rates to warrant their continued application.

At the beginning of World War II commercial vaccines were already available for immunization of American troops who might be sent into areas where the risk of infection existed. The question at issue was whether the strains of cholera vibrio composing these vaccines were the best possible ones for purposes of immunization. In general, old strains tend to undergo certain alterations that may affect their immunizing value. A program was therefore set up for studying a large number of strains of cholera vibrio, in order to discover which of them were best suited for use in a vaccine and to ascertain the antigenic relationships of these strains, so that the widest immunologic coverage of the total group might be represented in the vaccine. The problem was one of great complexity, and so much time was required for its solution achieved. However, much new information was acquired and new approaches to the objective were developed.

One approach to the determination of the immunizing efficiency of cholera vaccines is the analysis of the complex of antigenic substances contained in the cholera vibrios, some of which may be of greater importance than others. Previous work had shown that two types of antigen are present, one the heat-stable somatic or O antigen and the other the heat-labile flagellar or H antigen. The latter type is common to a wide variety of vibrios, including both cholera vibrios and non-cholera vibrios, such as water vibrios and the like. Several immunologic groups may be distinguished on the basis of the character of the O antigen. All the cholera vibrios and certain of the El Tor vibrios fall into a single group designated as O, group I. Japanese work had indicated that three immunologic types of cholera vibrio occur within this single group; these have been designated as the Inaba type, the Hikojima type, and the Ogawa type. Their immunologic structure has never been adequately defined.

The present study has indicated a multiplicity of antigenic components in both the somatic and flagellar portions of the vibrios, and suggests that for epidemiologic purposes detailed antigenic analyses should be carried out.

The relative importance of the above antigens as immunizing agents was studied with the use of a monospecific antiserum for passive protection tests that the practical application in the field of the results obtained was not

in mice. It was found that protection was associated exclusively with antibody to the O antigen, that to the H antigen showing no protective power. Absorption of all the agglutinating antibodies from antiserums left a residue of protective antibody that was attributed to the presence of an antitoxin, or perhaps more properly an antiendotoxin. The vibrio endotoxin was carefully studied with respect to its biochemical properties, pharmacologic action, and immunologic activity. Evidence was obtained that the toxin is nonprotein in nature and is associated with a phospholipid fraction of the vibrios. It could be extracted with various organic solvents such as methyl and ethyl alcohols, chloroform, and ether, but not with glycols. Purified material could be prepared that consistently killed mice in doses of 25 to 30 μg. The toxin appeared to be dialyzable through cellophane membranes.

The action of the toxin in the living animal was characterized by a marked alteration in capillary permeability, allowing extensive leakage of blood into the tissues. Intravenous inoculation in the rabbit's ear produced a large hematoma, and mice injected intraperitoneally showed extensive hemorrhage in the peritoneal cavity, but no other gross pathologic lesions.

It is usually assumed that in human cholera the vibrios establish themselves in the bowel, multiply rapidly, and then disintegrate and liberate endotoxin. Since the disease is characterized by a purging diarrhea and the pathologic picture is that of hypochloremia, dehydration, and an associated impairment of renal function, it seemed possible that the toxin might alter the permeability of the intestine to fluids. Accordingly, experiments were set up in which the passage of Ringer-Laski solution through living membranes, including frog skin, and the small intestine of the rabbit and the guinea pig, under low hydrostatic pressure, was measured. The rate of flow was accelerated in the presence of both crude and purified toxin. The acceleration could not be neutralized by antiserum, but tissue taken from actively immunized animals was almost completely resistant to the action of the toxin. These results suggest that antitoxic immunity may be of considerable importance in effective immunity to the disease, the functional antibody being present within the tissues of the intestine. The toxic agent appeared to be ten to twenty times more efficient as an immunizing substance than the intact vibrios themselves. These results indicate that an immunizing preparation to be of maximum efficiency must contain adequate amounts of endotoxin.

In order to study the character of immunity to infection with the cholera vibrio, an attempt was made to infect guinea pigs with the organism. An improvement was made in the method previously employed by Koch. The degree and progress of the infection were measured by the relative numbers of vibrios in the stools. Characteristic clinical symptoms were not produced. The differential pattern of vibrio excretion in the normal animal as compared with the immune animal has provided an extremely useful and sensitive measure of the efficacy of immunization. The relatively rapid disappearance

of the vibrios from the stools of immune animals has been associated with the presence of measurable amounts of antibody in the lumen of the bowel.

On the basis of the information gained from this study, a vaccine has been prepared and given preliminary trials in volunteers. The initial results show some promise from the experimental standpoint. A field trial would, however, be necessary in order to estimate what, if any, practical advantage the vaccine possesses.

A few chemotherapeutic tests of the activity of the sulfonamides and of streptomycin against cholera vibrios have been carried out. Sulfathiazole and sulfadiazine were the most highly protective of the sulfonamides in mouse-infection tests. Streptomycin, both in vitro and in similar infection tests in the mouse, seemed to be greatly superior to any of the sulfonamides.

This work has advanced knowledge of the antigenic structure of the cholera vibrio and has indicated methods for the selection of the most suitable strains for incorporation in a vaccine for use in the prophylaxis of cholera. The apparent importance of the endotoxin as an immunizing agent increases the probability of developing a vaccine that is more effective than any available in the past. The combination of preliminary active immunization with the therapeutic use of the appropriate sulfonamide drugs may enhance the total efficacy of methods of cholera control.

FOOD POISONING

Food poisoning or infection arising from food contaminated by certain types of bacteria, particularly the Salmonella group, presents a problem to the armed services because of the disabling effect of such an infection on a military organization. Epidemics resulting from this type of disease are explosive in character. They may be widespread, affecting all who have partaken of the contaminated food, and may thus interfere seriously with military activities at a critical time.

Epidemiologic control of food infection depends on the identification of the causative organism and discovery of its source. In order to achieve this purpose the intestinal contents of all food handlers must be examined bacteriologically, and persons recovering from the infection must be examined to establish their carrier status so that suitable controls may be established. In this way further dissemination of the infectious agent can be prevented.

The suspected organisms are identified by established bacteriologic technics and by specific serologic tests. To facilitate such procedures the Committee on Medical Research supported the preparation of a large number of different types of antiserums specific for members of the Salmonella group of bacteria. In addition, thousands of Salmonella cultures from the United States and abroad have been examined and each organism has been typed serologically. Many new species not previously recognized have been found. The specific

antiserums were sent to the Army Medical School for distribution, and Army and Navy personnel were trained in the diagnostic procedures involved in the study and control of Salmonella infection. In this way a basis was established for the understanding and limitation of food infections of the Salmonella type.

ACUTE RESPIRATORY INFECTIONS

INFLUENZA

Among the common diseases that affect military organizations, acute infection of the respiratory tract is without doubt the cause of the greatest amount of disability and of a high rate of ineffectives. At times and under certain conditions mortality rates due to respiratory disease may reach a very high level. New recruits are particularly susceptible to this type of infection, and from the very beginning of the period of mobilization more or less widespread epidemic outbreaks are the rule.

Of the acute respiratory diseases, the one that may attain the highest incidence and cause the greatest amount of disability and the largest number of deaths and that is in all respects the most alarming is epidemic influenza. Memory of the great pandemic of influenza that occurred toward the end of World War I and realization of its disastrous effects stimulated, at the very beginning of mobilization, the investigation of all possible methods for the control of this disease. Before the outbreak of the war, the virus etiology had been established, and methods of cultivating the virus in suitable amounts and preparing it in purified and concentrated form for use as a vaccine had been developed. Further improvement in technics was desirable, however, and a more thorough testing of the vaccine's efficacy as a preventive was necessary before a recommendation for its general use could be justified. Much of this work was carried out under the direction and control of the Office of the Surgeon General of the Army. In addition, the Committee on Medical Research inaugurated certain studies in the field of influenza.

Preliminary work consisted of studies of the conditions necessary to secure an optimum production of influenza virus in embryonated chicken eggs, of methods for the accurate titration of the virus, and of the effect of various chemicals on its activity. Virus preparations were examined in the ultracentrifuge and in the Tiselius electrophoresis apparatus. It was found that the sedimentation constant of different virus preparations varied with the concentration. By means of the electronmicroscope the virus particles were shown to be spherical; hence, the peculiar sedimentation behavior could not be due to asymmetry, as was found to be the case for certain other viruses. The difficulty was largely eliminated by the discovery that the virus prepa-

rations contained an impurity of low molecular weight with a very high viscosity and electrophoretic mobility. This impurity could be removed by means of fractionation in the centrifuge or in the electrophoresis apparatus. The preparations thus obtained were found to be homogeneous. Extensive biochemical, biophysical, and immunochemical studies were conducted with these highly purified preparations. The studies indicated that the particles, which were about 100 μv in diameter and consisted of protein, nucleic acids, lipid, carbohydrate, and about 60 per cent water, actually represented the influenza virus, in spite of the fact that they contained an antigenic structure characteristic of the host from which the virus was obtained.

Additional proof of this belief was provided by the concentration and purification of virus from infected mouse lungs. The biochemical and biophysical properties of this material were found to be essentially identical with those of virus obtained from chick embryos. However, the mouse-lung virus did not give a precipitin reaction with antiserum to normal chick embryo material but did show a relationship to normal mouse-lung material. Detailed quantitative immunochemical experiments were made, and the results of these have forced the conclusion that the 100-μu virus particles, regardless of their source, possess a common antigenic structure characteristic of the influenza virus, but possess in addition an antigenic structure characteristic of the host from which the virus is obtained. This conclusion if correct will introduce a new concept of virus structure. As a result of these studies, attention was directed to devising technics for concentrating and purifying the material on a large scale and to developing methods for using it in the preparation of a vaccine.

An evaluation was made of different methods of concentrating and purifying influenza virus, and it was found that centrifugation in a Sharples centrifuge was superior in certain respects to methods involving the adsorption by and elution of virus from chicken red cells, its elution from the precipitate formed on the freezing and thawing of infectious allantoic fluid, or the precipitation of the virus on calcium phosphate. In addition, the maximum concentration of virus obtainable by the centrifuge method was many times that obtainable by any other method, so that for large-scale concentration and purification of influenza virus this procedure has great potential usefulness.

The variable results obtained in early work on influenza vaccines were probably due to differences in the actual amount of virus present, in the degree of destruction of immunizing potency on inactivation of the virus, and in the ratio of virus to extraneous material. It seemed advisable to seek a vaccine purified so that the virus represents the major antigenic component, concentrated so that the virus concentration is several times that present in allantoic fluid, and inactivated in a manner that causes no loss in immunizing potency. Extensive studies were therefore conducted on the preparation

and properties of influenza virus vaccines concentrated and purified by cen-trifugation.

Various methods of inactivating the virus and stabilizing the immunizing potency were evaluated. Eventually a method was developed that yielded a vaccine possessing the sought-for features. The actual recovery of virus in the concentrate is usually about 80 per cent of that in the starting material. Centrifugation in the Sharples centrifuge serves to free the virus from most of the egg proteins, inorganic and organic salts, and other material rendered insoluble during high-speed centrifugation, as well as any residual bacterial contaminants that may be present. Bacteriostatic or bactericidal conditions are maintained from the time the extraembryonic fluids are harvested to the time the finished vaccine is bottled. The extraembryonic fluids, which have a protein content of less than 10 per cent of virus protein and over 90 per cent of non-virus protein, are converted into a product consisting mainly of virus with an over-all yield approaching 80 per cent. The extent of concen-tration possible is almost unlimited.

Clinical tests on the first two lots of vaccine prepared commercially by the methods developed under the auspices of the Committee on Medical Research were carried out in 1944. The antibody response and reactions fol-lowing doses of vaccine ranging from 0.01 to 10 mg. were determined, and the results indicated that with increasing size of dose up to about 2 mg. there was an increase in the neutralizing antibody titer and persistence of that increase with time and with the intensity of the reactions. The antibody response to 10-mg. doses was not much better than that obtained with 2-mg. doses, but the reactions were much severer. It became necessary to select a dosage level that would provide an antibody response adequate to ensure protection in the majority of persons and yet would not cause unduly severe reactions.

At the request of the Director of the Army's Commission on Influenza, a set of specifications on the centrifuge method was drawn up, and in April 1945 this method was accepted by the Army as a suitable alternative for the red-cell absorption and elution method that had been used exclusively during the preceding year. The manufacture of vaccine by the centrifuge method for the Army was begun near the middle of 1945, and early in 1946 the manufacture and distribution of centrifuge-type influenza vaccine for civilian use were started. As a result, it seems likely that means are now available for preventing influenza epidemics. Thus, the work on influenza virus and vaccines carried out with the support of the Committee on Medical Research and under other auspices has added much to our store of knowl-edge and has placed in our hands the means for preventing the influenza virus from again assuming the disastrous role played by it in the past.

Investigation of another method of controlling influenza virus infection was supported by the Committee on Medical Research. Some years ago it

was shown that the presence of a virus in living tissue could interfere with the development of another closely related virus if the first virus was inoculated shortly before the second. This phenomenon is now well established. Further work has shown that in some instances interference of the character described is manifested even when the interfering virus is not living but has been inactivated by chemical or physical means. The possible usefulness of this phenomenon in influenza has been investigated, and it has been demonstrated that if inactivated influenza virus is inoculated into the embryonated egg and the egg is subsequently injected with living virus, the latter fails to develop. Some claims for the usefulness of this principle in man had already been made. When, however, inactive influenza virus of an appropriate character was sprayed into the respiratory tracts of human beings during an epidemic of influenza, no benefit was derived. Whether further development of this procedure will prove efficacious is a matter for future investigation.

Primary Atypical Pneumonia

In 1936 or shortly before, an unusual type of bronchopneumonia appeared among the civilian population of the United States. The question whether this was a new disease was raised at that time. Whatever the answer might be, there could be no question as to the greatly increased detection of this type of pneumonia. By the time of mobilization the disease had become widespread and ranged in character from extremely mild to extremely severe manifestations. The mortality, however, remained low. This disease was officially designated by the Office of the Surgeon General of the Army as primary atypical pneumonia. It quickly became a subject for investigation by the Army and by many laboratories and medical schools throughout the country. Complete clinical criteria for the characterization and differentiation of this illness from other forms of pneumonia had not been established, and there were no known laboratory procedures that were helpful in making a positive diagnosis. Considerable numbers of cases of primary atypical pneumonia were being reported among the armed forces, as well as in the civilian population.

Investigation of this disease under the auspices of the Committee on Medical Research was under way early in 1942. At that time, knowledge concerning its cause was fragmentary and incomplete. Some workers suspected that the disease might be caused by a virus, but three distinctly different infectious agents, of which two were thought to be viruses and one a rickettsia, had been reported as associated with the illness in different small groups of cases. The role of bacteria as possible causal agents was still unknown, nor was there any knowledge of the factors responsible for susceptibility or resistance to the condition. Similarly, there was no information on the

mode of transmission of the infection from one person to another. There was evidence that sulfonamide drugs were ineffective in the treatment of the disease, but no effective treatment had been developed. There was no information concerning measures that might be useful in controlling outbreaks.

If primary atypical pneumonia became more prevalent, as seemed not unlikely under wartime conditions, its depredations might cause a considerable loss in efficiency among both civilians and armed-force personnel. Because so few facts regarding the illness had been definitely established, it appeared to be necessary to undertake a comprehensive and detailed study of the disease.

Investigations were carried on continuously for a period of forty-seven months. The major problems studied were the following: the clinical manifestations as well as the roentgenologic and laboratory features of primary atypical pneumonia observable in patients with the disease; the recognition and differentiation of primary atypical pneumonia from other forms of pneumonia, caused by either bacteria or viruses; the effectiveness of various forms of treatment; the role of various bacterial species and viruses as possible causal agents; and the development of reliable laboratory tests that would be useful in establishing a positive diagnosis.

Comprehensive clinical, roentgenologic, and laboratory investigations were carried out on more than 175 patients with primary atypical pneumonia admitted to the hospital of the Rockefeller Institute for the purposes of this study. In addition, analogous laboratory studies were carried out with specimens obtained from more than 150 similar patients admitted to other hospitals. The results of these studies indicate that primary atypical pneumonia can be recognized as a clinical entity on the basis of a number of fairly characteristic diagnostic criteria, but that it cannot be identified with any degree of certainty unless numerous laboratory tests are carried out. The validity of the diagnosis is dependent on the exclusion of a number of specific disease entities, caused either by bacteria or by viruses, any one of which may be associated with pneumonia. Infections induced by different disease agents may simulate closely the clinical pictures commonly seen in patients with primary atypical pneumonia.

Sulfonamide drugs are not effective in the treatment of this disease; they appear not to diminish its severity nor to shorten the course of the infection. The administration of penicillin in the usual dosage also appears not to alter significantly the course of the disease. Penicillin in massive dosage is still to be tested.

Detailed bacteriologic studies were carried out on various specimens obtained from each of the patients with primary atypical pneumonia admitted to the Rockefeller Hospital. It was found that none of the many bacterial species known to be associated with other forms of pneumonia were causally related to this disease. A hitherto unrecognized species of nonhemolytic strep-

tococcus, now designated as streptococcus MG, was discovered in the lungs of a number of patients who had died of the disease. This micro-organism was often present in large numbers in such lungs, and was present also in secretions obtained from the respiratory tract in many nonfatal cases. All strains belonged to a single immunologic type. Extensive serologic studies showed that a type-specific antibody response against streptococcus MG develops in a high percentage of patients with primary atypical pneumonia, and that a similar antibody response does not occur following a wide variety of other acute infections of the respiratory tract, including other forms of pneumonia. The extent of this response appears to be directly related to the duration of the manifestations of the disease. Antibodies directed against streptococcus MG are distinct and separable from the component in serum that is responsible for cold hemagglutination, and the two phenomena appear to be unrelated. The results obtained with streptococcus MG have been confirmed in other geographical areas by those noted by workers in a number of laboratories.

In order to gain more information concerning the role of streptococcus MG in primary atypical pneumonia, a study was made of its antigenic structure as well as that of other nonhemolytic streptococci. It was discovered that streptococcus MG and certain other nonhemolytic streptococci elaborate capsular polysaccharides, and that these substances are responsible for the type-specific immunologic reactions obtained with these bacteria. It is now possible to identify readily and to classify numerous nonhemolytic streptococci according to their antigenic structure. The capsular polysaccharides of streptococcus MG and two other types of nonhemolytic streptococci were separated from the bacterial cells and purified. It was shown that in primary atypical pneumonia antibodies are produced against the polysaccharide of streptococcus MG, but not against similar polysaccharides from other nonhemolytic streptococci. The purified polysaccharide of streptococcus MG, when injected into normal persons, stimulates the production of antibodies identical in both specificity and concentration with those that develop during primary atypical pneumonia. The antibody response that follows a single injection of the bacterial polysaccharide remains undiminished for at least seven months. A projected field trial of the possible efficacy of immunization with this polysaccharide antigen as a prophylactic measure against this disease was interrupted by the termination of the war and demobilization.

Extensive virologic studies were carried out on many specimens obtained from patients with primary atypical pneumonia admitted to the hospital, and also on specimens from similar patients in other hospitals. Vigorous attempts were made to recover a virus from such specimens by means of a wide variety of experimental procedures in eleven different species of animals. Multiple specimens of serum from many patients were tested against all viruses known to be capable of inducing respiratory infections in

man, as well as against other viruses suspected of possessing such capacities. These studies failed to yield direct, reproducible, and conclusive evidence either that a previously undescribed virus was causally related to primary atypical pneumonia or that any of the viruses tested were implicated in the pathogenesis of the disease.

During the course of the virologic studies it was found that certain latent viruses can and frequently do induce acute respiratory disease in various species of laboratory animals harboring such agents. The animal species in which such latent viruses are present include all those that have been used in this and other laboratories in attempts to recover a virus from patients with primary atypical pneumonia. Various nonspecific stimuli, including procedures generally considered innocuous, are capable of unbalancing the equilibrium between animal host and latent virus. When such an equilibrium is upset, demonstrable infection of the host with the latent virus frequently develops. Wholly noninfectious materials can in this manner actually induce the development of acute virus infections of the respiratory tract in normal animals, and such infections are followed by the development of specific immunity to reinfection.

A particular latent virus, the so-called pneumonia virus of mice, has proved of the greatest interest in this connection and has made possible the development of satisfactory laboratory models of acute respiratory infections. Intensive study of the peculiar properties of this virus, particularly the basis for the hemagglutination phenomenon obtained with it, has yielded information that may be helpful in solving a number of problems concerning the pathogenesis of common acute respiratory infections in man, including primary atypical pneumonia.

Indirect evidence in wide variety suggests that in primary atypical pneumonia one or another virus may be the initial incitant, even though in this laboratory attempts to obtain direct evidence for this view have been consistently unsuccessful. The available evidence suggests that streptococcus MG, although probably not the primary incitant, is none the less implicated in the pathogenesis of this disease.

MINIMAL PULMONARY TUBERCULOSIS

Up to the present time it has been almost impossible to evaluate the future course of minimal tuberculous lesions found in routine surveys of apparently well persons. In the x-ray examinations in connection with military service, large numbers of such cases have been discovered. These fall into two main groups: cases in which a minimal lesion was discovered in the preinduction physical examination, with consequent rejection for military service, and those in which a minimal active lesion was missed at the preinduction examination or in which the subjects were inducted under the

impression that the shadows seen on the x-ray film indicated completely arrested lesions.

The Office of the Surgeon General, as a result of surveys, estimates that for every 1,000,000 men inducted, 3000 have minimal active tuberculous lesions of the lungs. The care and disposition of this large number of cases constitute a serious problem. Because of the paucity of information on the course of minimal pulmonary tuberculosis in Army personnel and the lack of criteria for handling such cases, a collaborative study was set up under the joint auspices of the Committee on Medical Research and the Office of the Surgeon General at Fitzsimons General Hospital in Denver, Colorado, and the Bruns General Hospital in Texas, both of which are tuberculosis centers for the Army. The course and prognosis of minimal pulmonary tuberculosis in men in military service are being studied, with inclusion of psychiatric analysis to determine whether emotional factors influence the outcome of the minimal lesion. Approximately 900 patients have been investigated, of whom 400 have been selected for thorough psychiatric evaluation. Because of the nature and behavior of this chronic disease, any short-term study would be unreliable, so that a follow-up of five or more years must be made in each case before dependable conclusions can be drawn. With this in mind, the continuation of the study has been assumed by the Army Medical Research and Development Board.

AIR STERILIZATION AND SANITATION

In recent years much attention has been given to the study of the question of air-borne transmission of certain infectious diseases. That these agents of disease, which gain entrance to the body by way of the respiratory tract, do so through the medium of the air is now generally accepted by investigators. Virulent infectious material is expelled into the air as small droplets during the act of talking, coughing, or sneezing. Infection of exposed persons may take place within a fairly limited area by inhalation of the particles in the moist phase. On the other hand, the droplets may dry rapidly and settle on the floor or on other exposed objects, from which they arise as dust when disturbed by air currents or by certain forms of manipulation such as sweeping or the shaking of contaminated bedclothing. Many studies have demonstrated that infective agents retain their virulence for relatively long periods of time in the dry state. If dust or other particles obtained from the neighborhood of patients suffering from certain types of respiratory infection are examined bacteriologically, they are found to contain extraordinarily large numbers of living pathogenic micro-organisms. These organisms resemble in type those associated with the disease from which the patient is suffering. Just how wide dispersion may be has not been determined, but there is no doubt that infective dust can travel considerable distances.

An immense reduction in the incidence of gastrointestinal diseases communicated through the medium of contaminated water and food has been brought about through effective sanitary control of these substances. This disease-preventing and life-saving procedure has given rise to the hope that similar results might be attained in the respiratory group of infections by means of some practical form of air sterilization or sanitation. Certain studies had already demonstrated that infectious agents suspended in the air, either in the moist or in the dry state, could be rapidly destroyed by certain physical and chemical agencies. The most important of these have been found to be ultraviolet light and germicidal mists containing a variety of chemical substances.

Respiratory diseases manifesting the characteristics and phenomena described are the greatest cause of disability and death among all the medical conditions that afflict military organizations. The Committee on Medical Research therefore decided to explore the further development and application of the known methods of air sanitation, with a view to their practical employment among troops as a means of curtailing infection of the respiratory tract. The method chosen was the testing of chemical agents dispersed in the air as aerosols.

From a number of chemical substances suitable for the purpose those chosen were propylene and triethylene glycol. Preliminary tests showed that when these chemicals were dispersed in high dilution in the air of small experimental chambers, pathogenic bacteria and viruses sprayed into the chamber were rapidly killed. Susceptible animals placed in the chamber and exposed to infection were completely protected by the presence of the germicidal aerosol, whereas control animals introduced in the absence of the aerosol readily succumbed.

Studies were made of the concentration of glycol in air necessary for germicidal activity, and animals were exposed to this and greater concentrations for long periods of time in order to determine toxicity. Triethylene glycol was found to be the most suitable of the glycols, dilutions of even 0.005 mg. per liter of air causing the immediate death of air-borne organisms. No toxicity was observed at this concentration, and no discomfort was experienced by human beings breathing the treated air. Bacteria suspended in moist droplets were more readily destroyed than were those in the dry state. Relative atmospheric humidities of 25 to 60 per cent were necessary for optimum activity. Under the conditions of these experiments no fire hazard was found to exist.

The application of these laboratory observations to the actual control of respiratory disease necessitated the development of a practical means of glycol introduction, methods of vapor distribution and control of concentration, studies of the behavior of vapors, and carefully controlled tests on the efficacy of the vapors in reducing disease in large groups of persons. Such studies

have been carried out, and it has been shown to be possible to install glycol-generating and distributing apparatus for large-scale operation.

Preliminary statistics on the control of air-borne infection by these methods seem encouraging. A reduction in the total bacterial contamination of the air and perhaps a significant reduction in the number of respiratory infections in areas where glycol vapor has been applied have been produced as compared with control areas. The procedures are most effective in relatively small closed areas. The larger the space, the more difficult it becomes to maintain optimum conditions for effective action. A number of different types of apparatus for distributing glycol vapors and controlling their concentration have been developed. Further study and improvement of technics are necessary before generally practical and effective procedures can be made available.

INFECTION WITH HEMOLYTIC STREPTOCOCCUS

Hemolytic streptococcus infections may well be considered occupational diseases when contracted by recruits housed in barracks and cantonments while in training for active combat duty. Among these diseases, scarlet fever and tonsillitis are the outstanding and easily diagnosed examples. During World War I, much illness, characterized by severe and often fatal pneumonia, was induced by streptococci. Between the two world wars, it became increasingly probable that most cases of rheumatic fever are preceded by hemolytic streptococcus infection. During World War I, at least 20,000 cases of rheumatic fever were reported in the armed forces of this country. At that time it seemed that most of these cases occurred among troops who were living under environmental conditions favoring the transfer of infectious agents from one person to another; troops in the field and in the trenches, on the other hand, had a much lower incidence of rheumatic fever. Whether the large amount of nephritis seen during that war was secondary to hemolytic streptococcus infections was never accurately determined.

Among diseases of possible or probable streptococcal etiology encountered in the United States Army during World War I (from April 1, 1917, to December 31, 1919) in Europe and the United States, the following numbers of admissions are reported: valvular heart disease, 16,850; acute articular rheumatism, 23,818; muscular rheumatism, 11,328; scarlet fever, 11,189; erysipelas, 2426; acute nephritis, 2002; and chronic nephritis, 2958. One can only speculate as regards the relative importance of streptococci in the causation of the 164,192 cases of acute tonsillitis and the 36,012 cases of otitis media.

In the two decades prior to the onset of World War II, knowledge concerning hemolytic streptococci had been greatly expanded, and it had been thoroughly established by means of immunologic procedures that there are

many different kinds of hemolytic streptococci. It is fundamental to reliable epidemiologic studies that accurate identification be made of strains of streptococci. These micro-organisms are primarily divisible into large groups, the members of each group giving a specific immunologic reaction with group-specific serums. The streptococcal diseases peculiar to human beings are largely caused by members of so-called group A, whereas the diseases of lower animals are largely the result of infections with members of groups B and C. There are, however, enough exceptions to these rules to make it advisable, when investigating streptococcal diseases, to determine accurately the group to which any strain of streptococcus belongs.

Group A is made up of many different types, which have been identified by two different immunologic procedures: a slide agglutination technic, developed in England; and a precipitin test, developed at the Rockefeller Institute. In 1941 there was still considerable discussion as to which method would give the most satisfactory results and be most applicable in field studies. The slide agglutination technic had the advantage of requiring only small amounts of serum and culture; its disadvantages lay in the difficulty of having cultures in suitable condition for the test and in the variability of results reported by different observers. Reliable results were largely limited to highly trained workers.

It was believed that a precipitin technic would yield more reliable results in that the streptococcal component responsible for the precipitin reaction — the so-called M antigen — runs hand in hand with the virulence of the streptococci, and because the antibody against this substance is responsible for protection. It had been shown, on the other hand, that the antigenic substance or substances involved in the agglutination test might be either the above-mentioned M antigen or a second antigen, the so-called T substance. The latter has no apparent relation to virulence, nor has the antibody against it any relation to protection by immunologic means.

As studies of the antigenic components of group A streptococci progressed, it was shown that although each type had its own particular type-specific M substance, in certain instances members of several different types possessed related T substances. In only one instance was there an exception to this rule; namely, types 10 and 12, which because they have a common M antigen but distinct T substances are now designated as type 12. On the other hand, it was eventually established that types 15, 17, 19, 23, 30, and 47 have common T antigens, and that a similar condition exists among types 4, 24, 26, 28, 29, and 46, which have related T antigens different from those of the first-mentioned series. It is thus easy to understand how a given strain might be classified as type 4 in one laboratory by the slide agglutination test and as type 24 in another, because the T substance elaborated by this particular strain might have been detected with type 4 agglutinating serum in the first laboratory and by type 24 serum in the second. Another possibility existed:

the agglutination in one of the two instances might have been due to an M-anti-M agglutination reaction that would be specific for that particular type. It was only by analyzing these possibilities that the riddle of confusing reports could be solved.

Because the type specificity obtained with the anti-M precipitin technic seemed to be more closely related to factors of epidemiologic and immunologic significance, it was obviously important to modify this technic so that it was applicable to field studies. In 1941 extensive field studies were not practical because only twenty tests could be performed with each cubic centimeter of serum, and 150 to 200 cc. of broth culture of each streptococcal strain under consideration was required. After many trials, it was found that by making the tests with capillary pipettes 1 mm. in diameter, 1 cc. of serum was sufficient for one hundred and fifty to two hundred tests, and that the amount of broth cultures required was also much less. Arrangements were made for the manufacture of capillary tubing in sufficient amounts to make it commercially available, and other simple apparatus was developed. Incidentally, this capillary pipette technic has been adapted to several other immunologic investigations.

With the impending probability of war and the consequent danger from streptococcal diseases in the armed forces, the Rockefeller Hospital in the spring of 1941 increased the production of streptococcal grouping and typing serums in order to be partially ready for possible demands. Although types 1 to 30 (of which four are not members of group A) had been identified, many strains in the Rockefeller Institute collection could not be classified. With extended facilities made available early in 1942 by funds from the Office of Scientific Research and Development, many more rabbits were immunized with these previously nonclassified strains and eventually sixteen more types were identified, bringing the total number to forty-six. It was shown, however, that most of the streptococcal epidemics among the armed forces in this country were due to members of only about ten different types, so that the identification of new types has been less important than the furnishing of grouping and typing serums to various laboratories. Indeed, shortly after the capillary grouping and typing method was developed, requests for these serums became very numerous. By December 1943, diagnostic serums had been furnished to twenty-eight laboratories; among these were four naval laboratories, eight Army Air Forces laboratories, and three laboratories used by Army commissions.

The streptococcal typing in the Navy was largely concentrated in the Naval Medical School in Bethesda, Maryland. In the Army Air Forces, on the other hand, the work was done first in eight different laboratories by medical officers trained at the Rockefeller Institute; eventually many more were established, so that it was planned by V–J Day to have facilities for

grouping and typing streptococci in at least thirty Army Air Forces laboratories. The Army Commission on Respiratory Diseases and the Commission on Air-Borne Infections were supplied with all necessary serums for their studies of streptococcal infections.

Late in 1943, commercial production of these serums for the Army and the Army Air Forces was begun, but serums were thus produced for only sixteen types. A commercial organization under contract with the Office of Scientific Research and Development therefore continued to supply the remaining typing and grouping serums for these laboratories, as well as all the serums used in the Navy, until the summer of 1946. The total quantity distributed to various laboratories during the life of the contract was approximately 2700 cc. of grouping serums and 11,500 cc. of typing serums, representing a cash value of at least $35,000. All the serums produced by this company were also tested before being accepted for distribution to various Army laboratories.

Early in 1944, Dr. Stuart Elliott of England joined the Rockefeller Institute staff. He discovered that the difficulty in typing many strains of group A streptococci was due to their ability to elaborate a proteolytic enzyme that under ordinary conditions of cultivation destroys the M antigen on which the typing reaction depends. The production of this enzyme by a given strain of streptococcus seems to be inversely proportional to the virulence of that strain. In other words, a virulent strain (which produces much M substance) makes little if any of this enzyme; an avirulent strain makes much. It thus appears that another test may be available for distinguishing virulent from avirulent strains.

While this study of the antigenic structure of hemolytic streptococci was progressing in the Rockefeller Hospital, simultaneous investigations of streptococcal diseases were being conducted in the wards and contiguous laboratories of that institution and in neighboring naval organizations; hence, there was a constant clinical check on the usefulness of the methods as they developed. For example, this group of workers was the first to show that an actively progressing epidemic of respiratory streptococcal infections could be quickly terminated by the administration of 1 gm. of sulfadiazine daily to each of the exposed population. It has been further shown that diminution occurs in the virulence and in the M antigen content of the streptococci obtained from many patients as they become subacute or chronic carriers of these micro-organisms.

As a result of these parallel and closely related studies, technical difficulties were anticipated as they arose in more distant areas. Much time was devoted to teaching others the technics developed at the Rockefeller Institute. By reason of giving instruction in technics, furnishing serum to other laboratories, and classifying strains of streptococci sent from other activities, the

Rockefeller Hospital became the center through which flowed information gathered in various military and civilian laboratories, thence to be relayed to interested investigators.

The probability, anticipated early in the war, that streptococcal respiratory infections and the sequential rheumatic fever would prove to be serious and widespread diseases of military personnel was fully realized, but it can be stated that never before has the epidemiology of these diseases been so accurately described. This work was made possible by the technics and materials furnished under this contract and was based largely on the fundamental knowledge of streptococci developed by Dr. R. C. Lancefield and her coworkers. The possibility of making these materials available stemmed in part from grants from the Office of Scientific Research and Development, in part from facilities furnished by the Rockefeller Institute, and in part from the efforts of additional workers in the laboratory.

To attempt to cover all the investigations of streptococcal diseases in the armed forces is beyond the scope of this review, but some of the outstanding accomplishments may be outlined. In the first place, the nature of such epidemics was more accurately defined; for example, the occurrence in a given population of several cases of streptococcal infection, each due to a different type of streptococcus, should not be described as an epidemic. It was found, on the other hand, that a comparable number of cases all due to a single type of streptococcus in a similar population should be defined as an epidemic, because such a condition indicated the presence of a highly invasive strain.

It has been possible to determine that a variety of clinical conditions occur during a single epidemic of streptococcal infection; these conditions include scarlet fever, tonsillitis, nasopharyngitis, mild catarrhal fever, and even infections so mild that their existence can be established only from bacteriologic and immunologic data. All these clinical conditions can be induced by a single type — probably by a single strain — of streptococcus. The role of carriers in the spread of epidemics was more accurately outlined than had previously been feasible. The possibility was established of using sulfonamides to control epidemics, provided that the epidemic-inducing strains were susceptible to these drugs. Later this method of prophylaxis was shown to be useless in epidemics in which sulfonamide-resistant variants of a given type were disseminated through a susceptible population.

The sequence between group A streptococcus infection and rheumatic fever was established on a statistical basis that leaves little doubt of the close relation between the two, so that one can safely assert that in the absence of streptococcal infection rheumatic fever is quite unlikely to be present. Indeed, it was repeatedly demonstrated that the cutting short of an epidemic of streptococcal infection by chemoprophylaxis resulted in a corresponding falling off of new cases of rheumatic fever. It therefore appears that the

occurrence of rheumatic fever and rheumatic heart disease is related to certain geographical areas, housing conditions, and occupational environments favoring the dissemination of streptococcal diseases. It has been shown that rheumatic fever may be induced by many different immunologic types of group A streptococci, and that the precursory streptococcal infection may be so mild as to escape clinical detection. Likewise, the rheumatic manifestations following a streptococcal infection may be largely limited to the heart and be demonstrable only through the correlation of exact clinical, laboratory, and electrocardiographic data.

The analysis of the antigenic components of streptococci not only revealed certain principles applicable for future studies of streptococcal infections but was immediately instrumental in supplying technics and materials with which accurate investigations of these diseases could be made, especially in the armed forces. Although scarlet fever and streptococcal respiratory infections are no longer the immediate death-dealing diseases they once were, it is safe to say that they remain high in the list of maladies that lead to chronic disabling infirmities and eventually to fatal outcome after years of economic dependence. The results of investigations in the armed forces conducted under contracts with the Office of Scientific Research and Development can therefore be transferred directly to civilian public-health practice.

The manufacture and distribution of streptococcal grouping and typing serums by a privately endowed research institute can be justified as a war-emergency measure, especially when public funds are provided to further this effort. Such functions, however, cannot be expected to continue in peacetime. The cost of producing these serums will not be borne by commercial laboratories unless there is the prospect of at least a small profit, or reassurance that a deficit will be subsidized by some governmental or private agency. At the present moment no such reasonably permanent prospect is in sight. The possibility, or even probability, must therefore be faced that there will be little long-term extension under civilian auspices of much of the work herein described, because some of the materials for carrying out such investigations will not be readily available. Unfortunately, the possession of antibiotic agents dulls the demands of society, both lay and professional, for the investigation and control of diseases that are not immediately death-dealing or obviously crippling. Hemolytic streptococcus infections are such diseases. How they might be studied was well illustrated by the various workers in the armed forces. With the diminution in recruiting and the return of medical officers and bacteriologists to civilian life, the demand for these investigations has dropped precipitously. Although scarlet fever and streptococcal respiratory infection still take their annual toll in the form of thousands of cases of rheumatic fever and eventually of rheumatic heart disease, there is as yet little community effort comparable to that in the armed forces to study how these maladies may be prevented.

Possibly some of the temporary military investigators will want to carry this type of study over into civilian public-health activities after returning to their homes; but this desire will be thwarted and the benefit of their special training largely lost unless some agency, either privately or governmentally supported, makes possible the continued utilization of the required technics and materials. How the efforts of government agencies, private institutions, and military authorities were combined for the solution of some of the problems imposed by streptococcal diseases is well illustrated by this study. How comparable efforts can continue to be applied both to military and civilian public-health problems in peacetime is a question worthy of pursuit, for in its answer probably lies the solution of the prevention of rheumatic fever.

ENCEPHALITIS

Since 1933, epidemics of the St. Louis type of encephalitis have occurred in man, and since 1938 infection with both the eastern and western types of equine encephalitis has also been recognized in human outbreaks. Horses are affected yearly in every western state, and much of the central area east of the Mississippi River and occasionally East Coast areas are also involved. These types of encephalitis are due to infection with a filtrable virus.

The age group usually affected in the West is that of young adult workers, particularly those engaged in outdoor occupations. In the area of the Dakota outbreak in 1941 about 3000 human cases were recognized, and other outbreaks of western equine, eastern equine, and St. Louis types occurred in Montana, California, Washington, Arizona, New Mexico, and Texas. In Manitoba, Canada, military personnel contracted the disease, but in the United States only 4 proved cases occurred in the military forces.

In view of the large concentration of troops in the regions mentioned and of the possibility of the recurrence of serious outbreaks of the disease in areas of military occupation, the Committee on Medical Research undertook to contribute to the study of the epidemiology of encephalitis. Various species of Aedes mosquitoes had been found in laboratory studies to transmit the viruses of the equine encephalitides to experimental animals. The mosquito *Culex pipiens* is capable of transmitting St. Louis encephalitis to laboratory animals, and *C. tarsalis* had been found in the Yakima Valley of Washington State to be infected in the natural state, in 3 cases with the St. Louis encephalitis virus and in 5 with the western equine virus. *Triatoma sanguisuga* was found naturally infected in Kansas but appeared to be a poor transmitter. *Dermacentor andersoni* transmits the equine type and *D. variabilis* the St. Louis type in laboratory experiments. In the Yakima Valley, 50 and 35 per cent of domestic animals and birds and 20 and 8 per cent of wild species, respectively, showed serologic evidence of infection. Similar figures

were obtained for areas in the California Valley. In view of these facts, a thorough study was undertaken of the role of arthropods, particularly mosquitoes, in the transmission of the western and eastern equine and St. Louis types of encephalitis.

Studies in the Yakima Valley had focused attention on *C. tarsalis* as a possible vector of western equine and St. Louis viruses. To strengthen the case against this mosquito, proof had to be obtained that once infected it could transmit each of the viruses by biting. In order to test the role of domestic animals and fowl as reservoirs for mosquito infection, it was necessary to demonstrate that some of these species following peripheral inoculation of the virus could serve as sources of mosquito infection. Likewise it was necessary to show that following the bite of an infected mosquito, virus was present in the blood stream of the above species. Since in other epidemic areas other arthropods with different feeding habits might serve as vectors of the virus, it was important to make comparable studies in those regions. At the same time, tests had to be made of a number of species of mosquitoes from each region for their ability to serve as vectors in laboratory experiments under such conditions of temperature and humidity as are observed in nature. Thus a pattern for an epidemiologic study was developed whereby, with only slight modification in any region where an insect-borne virus encephalitis occurs, the most important epidemic vector or reservoir of infection can be determined. Control measures may then be directed against either vector or reservoir or both, as circumstances indicate.

Members of the research unit made field epidemiologic studies of human and horse epidemics in California, Washington, Arizona, Texas, Oklahoma, and Nebraska. Certain of these investigations were made at the request of the Army and the Navy and others at the request of civilian groups.

During the studies mosquitoes have been proved to be important vectors of the encephalitis viruses. Over 195,000 blood-sucking arthropods were tested for virus infection, and more than one hundred strains of western equine and St. Louis virus were isolated. The mosquito that appears to be the chief vector of the western equine and St. Louis types is *C. tarsalis*.

In the search for vertebrate reservoir hosts, more than one thousand serums from normal animals were tested for antibodies. In addition, laboratory studies were made to determine which animals can serve as a source of mosquito infection. It was demonstrated that domestic and wild birds serve as hosts for the disease organisms, although they show no signs of disease, and that they are important sources of mosquito infection.

The chain of infection for the encephalitis viruses is now believed to be that of bird to mosquito to bird, with the mosquitoes that commonly feed on birds also feeding on and infecting man and other animals. As a part of this study, investigations of the feeding habits of mosquitoes were made by the blood-precipitin test technic, and it was found that *C. tarsalis* fed

most frequently on birds but also fed on large mammals, including man.

Extensive laboratory studies were carried out to determine which species of mosquitoes are potential vectors of the domestic and foreign viruses of this group. A total of two hundred and twenty-five laboratory experiments were completed, as a result of which many mosquito species not heretofore considered to be of significance, except as nuisances, are now known to be capable of acting as vectors.

Studies were made to determine whether, should foreign members of this virus group (such as the Japanese B type) be introduced into the United States, they could become established here, with resultant epidemics. Seven of the commonest species of western North American mosquitoes were proved to be capable of acting as vectors of the Japanese B type virus, and several of the ordinary domestic animals were shown to be capable of serving as hosts. In case this virus, which causes high mortality rates in the areas where it now occurs, should be introduced into the United States, both suitable vectors and hosts have been shown to exist. After this work was taken over by the Army, field studies on the Japanese B virus were carried out in Okinawa and Japan.

Studies have been begun to develop methods for prevention of the diseases due to the various types of encephalitis virus by means of control of the significant species of mosquito. There is hope that suitable control technics can be developed, such as those that have already been applied in the control of such mosquito-borne diseases as yellow fever, dengue fever, and malaria. In fact, mosquito-control measures, in large part stimulated by these studies, may possibly be credited with the absence of large numbers of cases of encephalitis among troops.

PLAGUE

Bubonic plague due to infection with *Pasteurella pestis* is an extremely dangerous and in some instances highly communicable disease, which is endemic and frequently epidemic in certain eastern areas, such as China, India, Burma, and the Dutch East Indies. The bubonic and septicemic forms are communicated to human beings by the bite of the rat flea *Xenopsylla cheopis,* and the pneumonic form by direct contact with infected persons. Mortality rates have always been exceedingly high. In view of the probability that American troops would be operating in the areas of possible exposure mentioned, consideration was early given to an investigation of all known means of protection against infection.

Active immunization by the use of plague bacillus vaccines had been employed for many years and was believed to be of value in reducing both morbidity and mortality. The vaccines were of two types, those containing the organisms killed by heat and those containing attenuated living bacilli;

the former were preferred because of the potential danger in the use of living organisms. Anti-plague immune serum had been used in treatment of actual cases, but its therapeutic value had not been definitely established.

Study of published records dealing with the subject of plague vaccines disclosed a variety of views concerning their preparation and use and suggested the advisability of further investigation. Attempts to perfect a method of prophylactic immunization against plague are based on the observation that survival from the disease protects against a second attack or at least transforms such an attack into an infection with a milder course. The fact that suitable preparations of *P. pestis* may protect rats and mice against severe infection is considered presumptive evidence of their potential efficacy in human beings. Both the Haffkine type of vaccine — plague bacilli grown in broth and killed by heat — and the vaccine prepared either from agar-grown bacteria or bacillary fractions, such as the nucleoproteins, have been shown to have immunizing value for mice and rats. It was recognized, however, that these vaccines only exceptionally conferred immunity on the guinea pig, the animal most susceptible to experimental infection with the plague bacillus. Since the Haffkine and related heat-killed vaccines rarely protected guinea pigs and likewise often failed to reduce the death rate when used during human epidemics of plague, it was believed that a correlation existed between the protective value of the vaccine for guinea pigs and its efficacy for human beings.

In a search for better immunizing preparations, Strong and, more recently, Otten had found that avirulent living plague bacilli derived from attenuated cultures were highly protective in the guinea pig. Such living vaccines were also capable of reducing the fatality rate in human beings from 17.5 per cent to below 4 per cent. The use of live avirulent bacteria, however, involves some risk, particularly since two strains of the organism supposed to be avirulent have occasionally proved to be infective for the guinea pig. Furthermore, the conditions under which living plague vaccine had to be prepared in a central laboratory and distributed from there to the armed forces made the use of avirulent live vaccines inadvisable.

Research was therefore focused on the manufacture of vaccines prepared from formalin-killed organisms suspended in carbolized saline solution containing 0.5 per cent formalin and with a concentration of 2000 million plague bacilli per cubic centimeter of fluid. In co-operation with the United States Public Health Service this vaccine has been carefully evaluated by the mouse-protection test. Although this test detects only major differences in the immunizing capacity of different bacterial preparations, conclusive evidence was obtained that 2,000,000 to 3,000,000 killed organisms protected mice against infective doses of 2000 to 4000 virulent plague bacilli. Although the protective value of this vaccine for guinea pigs was low, by the utilization of a variety of methods a vaccine was finally obtained that protected 15 to

46 per cent of guinea pigs inoculated with living plague bacilli, whereas 90 to 100 per cent of control animals died. The immunizing potency of the vaccine was markedly enhanced by precipitation with alum, giving rise in some experiments to protective rates of 80 to 100 per cent.

These observations cast doubt on the claims of some investigators that active immunity in guinea pigs can be produced only by the injection of certain living avirulent strains of *P. pestis*. Furthermore, they definitely indicate that the antigenic structure is not injured by the physical and chemical agents employed, and that killed plague bacilli precipitated with synergistic substances, such as alum, protect the highly susceptible guinea pig against infection. Animal experiments also furnished convincing evidence that the degree of protection conferred on mice and guinea pigs is definitely raised when large and repeated doses of vaccines are injected prophylactically.

The above plague vaccine produces on injection into man local and systemic reactions of variable intensity but mild in comparison with those ordinarily induced by the Haffkine type of vaccine. The immune response in man was tested with the assistance of volunteers, and it was found that about two thirds of those inoculated showed evidence of immunity as determined by an agglutinin test. Approximately the same number developed in their serums antibodies with weak protective value for mice. Conclusive evidence has therefore been obtained that dead plague vaccines with proved immunizing potency for mice and guinea pigs are capable of inducing a measurable immune response in man. Large doses of the vaccine, containing 8000 to 12,000 million killed plague bacilli, and equivalent booster doses increase the percentage of positive responses and induce a higher level of antibody concentration in the serum.

In a preliminary series of human vaccinations with living avirulent plague bacilli, either the serum protective antibodies developed were of low concentration or their presence could not be demonstrated. The few tests thus far completed furnish no conclusive evidence that avirulent living plague vaccines are superior to killed vaccines when the appearance and concentrations of serum antibodies are used as a measure of effectiveness.

The above studies indicate that inoculation with agar-grown, formalin-killed, and partially detoxified plague vaccine should prove effective as a prophylactic against plague. The procedure should be employed before an outbreak of plague is in progress. The degree of protection afforded cannot be measured but is probably high, provided that inoculations of adequate dosage — 8000 to 12,000 million organisms — are given repeatedly and that equivalent frequent booster doses are also administered.

Early studies on the treatment of plague with anti-plague horse serum indicated that the administration of such a serum resulted in a reduction of the case fatality rate by not more than 7 to 10 per cent. Previous experiments

had also shown that immunization of species of animals other than the horse gave rise to antiserums of greater potency than could be obtained by the use of horses, and that rabbit anti-plague serum had an immune body titer many times that of a corresponding horse serum. These observations have been confirmed and extended in the present studies. By application of the newer methods of plasma fractionation and concentration, antibody solutions of high potency have been prepared. Satisfactory methods for titration of the protective value of anti-plague rabbit serums have been developed.

Since 1939, extensive studies of experimental plague infection in mice and guinea pigs and certain field tests on human plague have established the therapeutic efficacy of the sulfonamides in the treatment of plague infections. Sulfadiazine, sulfapyrazine, sulfamerazine, and sulfathiazole are all useful for this purpose. Of these compounds sulfadiazine is the drug of choice because of its lower toxicity and the easy maintenance of adequate blood levels. Previous inoculation with plague vaccine increases the likelihood of a favorable outcome in experimental animal infection with plague when the animals are concurrently treated with the above-mentioned sulfonamides. A limited number of observations indicate that the use of sulfadiazine may serve as a useful prophylactic measure for persons exposed to infection with pneumonic plague.

The combination of serum therapy with chemotherapy seems to double the therapeutic effect to be expected from each remedial agent used separately. Study of the treatment of human beings by the sulfonamides in combination with anti-plague serum, particularly the concentrated immune globulin fractions of rabbit serum, should be undertaken as soon as sufficient quantities of the latter are available. Methods of administration and dosages have not yet been determined.

Although penicillin exhibits no bactericidal or bacteriostatic action against *P. pestis,* streptomycin has proved remarkably effective in the case of experimental infections with this organism in animals. In vitro the latter antibiotic in a dilution of 1.9 μg./cc. inhibits the growth of the plague bacillus in broth culture for as long as six days. Cure of an advanced infection in mice can be attained in 100 per cent of cases by treatment daily with 500 to 1000 units of streptomycin administered every three hours, the equivalent of a daily dose of 4 to 8 mg., for three days. The results of the therapeutic experiments with this drug in plague-infected animals are sufficiently impressive to warrant clinical trials in human septicemic and pneumonic plague, in which the sulfonamides are known to be ineffective.

These studies, based to a certain extent on previous work but confirming and extending it, have laid a promising foundation for the development of better methods of control and treatment of bubonic plague. The combination of vaccination, serotherapy, and chemotherapy should result in a greatly decreased death rate from this highly dangerous disease.

GAS GANGRENE

In civilian medicine gas gangrene is a relatively infrequent complication of traumatic wounds, owing not only to the infrequency of contamination by soil containing the causative organisms but also to early surgical treatment involving débridement and cleansing of the wound. On the other hand, the history of warfare prior to World War II is filled with accounts of this most dreaded complication of wounding.

During World War I a considerable step forward in understanding the pathogenesis of gas gangrene was made with the discovery that the Welch bacillus, the chief etiologic agent, produces a potent exotoxin that is responsible for most of the serious effects of gas-bacillus infection, both local and systemic. At about the same time studies in England revealed the conditions under which gas-bacillus infections are most likely to occur. It was known that the intestinal tract of man and animals, as well as soil, contains the gas-gangrene anaerobes, but that contamination of clean wounds with the organisms alone does not suffice to establish infection. Crushing and devitalization of tissue, especially muscle, and deposition of soil in the wounded area set up conditions that permit germination of the spores of the gas-gangrene anaerobes, multiplication of the vegetative forms, and production of exotoxins with local and general tissue damage. The killing action of toxin is caused by absorption into the general circulation from the local area.

Of the constituents of soil, calcium was found to be the outstanding potentiating factor, and a technic of experimental infection was worked out in which the spores or vegetative forms of the gas-gangrene anaerobes, suspended in a solution of calcium chloride or a suspension of sterile earth, were injected intramuscularly in experimental animals. The necrotizing effect of calcium chloride or earth enabled the organisms to become established, and a well-standardized experimental infection could thus be used for study of the efficacy of prophylactic and therapeutic measures.

It was likewise shown that the so-called alpha toxin of the Welch bacillus, through its damaging local effect on tissues, is also an important potentiating factor in providing conditions favorable for the growth of the bacteria. Of the several exotoxins produced by this bacillus, the alpha toxin causes the most serious local and general effects. Similarly, the other principal gas-gangrene anaerobes produce exotoxins. Immunization of experimental animals with untreated filtrates of organisms grown in artificial media or with toxoids prepared by treatment of culture filtrates with formalin results in the production of antitoxic antibodies that neutralize the toxin and confer both active and passive protection against experimental infection with gas bacilli. Antitoxins prepared by the immunization of horses with toxins have been in use since World War I for the prevention and treatment of gas-bacillus in-

fections, in analogy to similar methods employed against tetanus and diphtheria, both of which are also caused by organisms producing exotoxins responsible for most of the ill effects associated with these infections.

The prophylactic effect of Welch antitoxin in experimental animals was well known, but in man the observations were insufficient to permit conclusions to be drawn concerning either prophylaxis or therapy.

In early experiments with the sulfonamides these drugs exerted a moderate therapeutic effect when given early. Applied locally to prevent infection they were likewise moderately successful. Consideration of the nature of the local lesion in gas gangrene, as compared with other bacterial infections, affords an explanation for the relative inefficacy of the sulfonamides. Gas gangrene occurs almost exclusively in areas of the body where damage to the circulation has occurred. The resulting partial or complete ischemia sets up conditions of oxidation reduction that permit germination of the spores of the gas bacilli and at the same time allow the infection to progress with little or no resistance by the general bodily defense mechanisms. The same conditions of ischemia and death of local tissue are strong limiting factors in the usefulness of antibacterial chemotherapeutic agents, whether sulfonamides or penicillin, since there is no assurance that the systemically administered drug will reach the involved area through the circulation in a concentration sufficient to exert an antibacterial effect. Moreover, since most of the ill effects of gas bacilli are due to the toxins produced by the organisms and not to the bacteria themselves, treatment of established infections with chemotherapeutic agents cannot be expected to be highly successful, since the drugs available do not exert an antitoxic action.

The probability that war in Africa and especially eastern Europe would be waged over areas known from the experience of World War I to be highly infested with the spores of virulent gas-gangrene organisms made it reasonable to assume that many cases of gas gangrene would occur among our troops. Fortunately, this expectation was not realized. The lesser number of cases in relation to the total wounded during World War II was almost certainly due to much earlier and more adequate surgical care in both American and British forces.

Although the incidence of gas gangrene was lowered greatly during this war, it should not be assumed that it has ceased to be a problem. There is good reason to believe that the progress already made, particularly through better surgery, could be greatly advanced from the standpoints of both morbidity and mortality were effective immunization procedures available. The virtual eradication of tetanus from our troops during World War II as a consequence of general immunization with a potent toxoid gives a clear indication of what might be accomplished by immunization against gas gangrene.

Because of the theoretical and practical shortcomings of chemotherapy for

either prophylaxis or therapy, the Committee on Medical Research embarked on a program of preparing toxoids from the exotoxins of the various gas-gangrene anaerobes for the immunization of troops. Experimental work demonstrated beyond question that if the toxins elaborated by the most important of the gas-gangrene organisms, the Welch bacillus, were neutralized by antitoxin, whether appearing as a result of active immunization or passively administered before infection, the infection remained sharply localized and the serious systemic effects due to absorption of the exotoxins did not appear. Active immunization of all troops was considered as offering much more hope of success than administration of antitoxin after wounding, since under battle conditions the giving of antitoxin is frequently delayed until infection has been established, in which event the therapeutic effect is limited.

At the same time, studies were set up on experimental infection of dogs with mixtures of the three chief species of gas bacillus. *Clostridium welchii, Cl. oedematiens,* and *Cl. septicum,* in order to assess in larger animals the relative potency of sulfonamides, penicillin, and antitoxin, used alone or in combination, as well as the prophylactic effect of active immunization with toxoids. These studies, which are described in the section on the surgical aspects of gas gangrene, demonstrated that active immunization of dogs with toxoids is highly effective in neutralizing the lethal action of the gas-bacillus toxins, although it does not prevent the development of the local lesion. These results in large animals confirmed earlier observations in guinea pigs and mice and provided further support for the view that active immunization of man can be expected to prevent the serious consequences of infection with the gas-gangrene anaerobes.

In order to prepare toxoids for human immunization, it was necessary to discover the conditions under which the organisms produced the highest yields of toxin when grown in an artificial medium. In the case of *Cl. welchii,* which is responsible for the great majority of infections, serious problems were encountered in obtaining yields of toxin high enough for the preparation of effective toxoids. It was found that although the organisms grow profusely in solutions of defined chemical composition, only very small amounts of toxin are produced under these conditions. Through this observation it was discovered almost simultaneously by both British and American workers that a factor or factors other than those required for growth of the organisms are necessary for toxin production.

In working out the role of these toxin-promoting factors, investigators in both countries followed similar paths. Attempts were made to improve toxin yields by adding to culture media varying amounts of materials found to contain the toxin-promoting factors, notably beef or horse muscle, pancreas, casein from milk, and a complex sugar, dextrin. In addition, studies on the isolation and purification of the toxin-promoting factors were vigorously

pursued, with the ultimate aim of being able to add to culture media the proper amounts of the various purified factors required for toxin production.

A practical culture medium was finally devised in which the toxin-promoting factors for *Cl. welchii* were supplied by pancreatic digests of casein or alcohol-extracted muscle, with the addition of dextrin. Active collaboration of pharmaceutical houses in the preparation of large lots and the production of formalinized toxoid from concentrated solutions of toxin placed in the hands of investigators materials that could be used experimentally for the immunization of volunteers. The same medium devised for preparing toxin from *Cl. welchii* was found satisfactory for toxin production by the other two important species of gas-gangrene anaerobes.

Trial immunization studies with volunteers, chiefly students in medical and dental colleges, with each of the three toxoids, separately and in combination, led to determination of the optimal amount of each required and established that two doses of alum-precipitated toxoid, the second injected three to four weeks after the first, give an immune response that on the basis of animal experiments might be expected to protect man against infection. It was also found that a single dose should be repeated within six months after primary immunization to ensure maintenance of antibodies in the circulating blood. The use of a booster dose greatly improved the immune response in almost all subjects tested; moreover, the amount of toxoid effective as a booster was much less than that required for primary immunization.

Reactions to subcutaneous injections of the gas-gangrene toxoids were observed to be no more marked than those associated with immunization to tetanus and of less severity than reactions to typhoid vaccine.

With the development of these immunizing preparations, the Surgeon General of the Army formulated plans for testing their efficacy in preventing gas gangrene in troops under battle conditions in the Asiatic Theater. Owing to the surrender of Japan, these plans were not carried into effect.

Studies on the nature of the toxin-promoting factors for *Cl. welchii* have shown that at least two and probably more distinct substances must be present for toxin production to occur. The number of factors involved makes isolation and characterization of these substances a complex and tedious pursuit, since the effect of any one of them cannot be tested separately but must be related to the quantity of the others present. Since the nature of none of these substances has yet been discovered, tests must be on a trial-and-error basis.

One of the next significant steps in gas-gangrene prophylaxis will undoubtedly be the preparation of the toxoids on a simple medium the constituents of which are known. By this means, present difficulties in obtaining uniformly high yields of toxin from one batch to the next can be avoided; moreover, the resulting toxoids will be free of contaminating material de-

rived from muscle, pancreas, or casein, which may give rise on occasion to undesirable effects on injection into man. Continuation of studies on the nature of the toxin-promoting factors for *Cl. welchii* is therefore highly desirable.

Until recently little has been known of the mechanism whereby the toxins of the gas-gangrene bacilli exert their poisonous effect, whether locally or systemically. Much light was thrown on this problem through the studies of a group of British workers who showed that the lethal toxin of the Welch bacillus is an enzyme that destroys lecithin, an important constituent of most of the tissues of the body. This discovery was of value not only because it permitted more rational analysis of the action of the toxin in the body, but also because it afforded means for devising a quick and accurate method for measuring in vitro the amount of toxin in any given preparation. Previous methods employed large numbers of laboratory animals, especially mice, with consequent limitations in the number of tests that could be carried out and with less accurate results than could be obtained by the in vitro method unless large numbers of animals were used. The employment of the latter method for measuring toxin assisted materially in the development of an effective toxoid for immunization against infection with *Cl. welchii*. In the case of the toxins produced by the other two important species of gas-gangrene anaerobes, however, reliance must continue to be placed on animal assay for measuring the amount of toxin that is present in a given preparation.

In the case of the Welch bacillus, most of the damage caused by the organism is due to the toxin secreted in the local lesion during growth, little damage being caused through general invasion of the body by the organism itself. *Cl. septicum,* on the other hand, tends to cause a generalized infection in experimental animals, and although antitoxic immunity is of definite benefit, its effect is by no means as striking as that achieved with *Cl. welchii.* It has been found that antibodies directed against the organism itself — that is, antibacterial antibodies — supplement strongly the action of antitoxin. Effective immunizing preparations against infection with *Cl. septicum* should therefore include antigens derived from the somatic portion of the organism itself, in addition to the toxoid prepared from its toxin.

The occurrence of a number of types of *Cl. septicum,* each having a different antigenic structure, although all produce an identical toxin, makes the practical solution of this problem difficult. It seems likely, however, that although the sulfonamides and penicillin are able to exert little effect on the local lesion once it has developed, both groups of drugs, especially penicillin, may be of considerable usefulness in preventing systemic invasion by *Cl. septicum.* The results of experiments in mice support this view, so that the employment of chemotherapy in persons immunized against the toxin may be expected to be more beneficial than either procedure by itself.

TYPHUS FEVER

The probability of exposure to epidemic typhus fever in certain areas of military operations made the perfecting of methods of control of this disease a matter of great urgency. In fact, a satisfactory and effective vaccine against typhus fever was rapidly developed by governmental agencies other than the Office of Scientific Research and Development. However, the latter agency co-operated with the Army in the study of technical methods for preparing typhus vaccine. The object of the investigation was to free the bodies of the rickettsia of typhus, grown in the yolk sac of eggs, from contaminating egg protein that might be present and to effect their further purification by physical and chemical means.

The rickettsial antigens were subjected to fractionation procedures that permitted the isolation of the typhus-specific substances. These purified antigens were found to compare favorably in immunizing value with the usual typhus vaccine containing the whole bodies of the rickettsiae. A soluble antigen present in supernatant fluid after removal of the rickettsial bodies by centrifugation was found to have significant immunologic properties. Study of the immunizing capacity of the various antigenic substances prepared was carried out by the Virus Laboratory of the Army Medical School in Washington.

A study was also made of the mechanism of the Weil-Felix reaction, a reaction helpful in the diagnosis of typhus fever. Chemical analysis of proteus OX19, the organism used in this test, revealed the presence of two antigenic components, one of which is responsible for the specificity of the test. The advisability of preparing this substance in purified form for diagnostic purposes in the identification of epidemic typhus fever is worthy of further consideration.

INACTIVATION OF VACCINES BY ULTRAVIOLET IRRADIATION

The Committee on Medical Research fostered the development of an improved method for the production of potent vaccines inactivated by ultraviolet irradiation. It was thought that a new method of destroying the organisms without the use of heat or chemicals might have less denaturing effect on the antigen. Ultraviolet light was selected for this purpose. After a large series of experiments and studies, it became apparent that there were fundamental conditions that must be observed in order to sterilize or inactivate infectious agents with a minimum of deleterious effect on the antigenic structure. It was learned that sterilization must be accomplished with extreme rapidity so as to avoid the production of heat in the suspension of

organisms. To accomplish this purpose, a special type of ultraviolet light was developed that was not only a powerful source of total ultraviolet rays but was largely composed of Schumann rays (1850–1220 Å); ultraviolet light in the Schumann range is particularly bactericidal.

Because ultraviolet light is readily absorbed, the film of a bacterial or virus suspension had to be extremely thin. Means were developed for constructing lamps that constituted a constant and powerful source of ultraviolet light and Schumann rays. Quartz irradiation chambers of extreme thinness were used, so as to permit a regulated flow of bacterial suspension and to keep the distance of the suspension from the lamp uniform and constant. All variables (ultraviolet energy output, film thickness of suspension, distance from ultraviolet source, and irradiation) were standardized and measurable, so that results could be duplicated.

The new method completely kills or inactivates turbid suspensions of bacteria and viruses in a fraction of a second. Micro-organisms with a film of less than 1 mm. are exposed by continuous flow to the action of the newly developed lamp, which is a powerful source of both total and extreme ultraviolet irradiation. Bacteria and viruses were rapidly inactivated by this technic, with a minimal loss of antigenic potency. The conditions of irradiation were standardized with respect to intensity, film thickness, and time of exposure so that experiments could be repeated with consistent results.

By means of the above technic, suspensions containing approximately one billion organisms per cubic centimeter of the following bacteria were completely killed in 0.17 to 0.33 second of exposure to ultraviolet rays: *Bacterium coli, Eberthella typhi* (strain 58), *Salmonella enteritidis, Staphylococcus aureus, Streptococcus viridans, Strept. haemolyticus, Diplococcus pneumoniae, Pasteurella pestis,* and various species and types of Shigella. Four per cent uncentrifuged brain-tissue suspensions infected with fixed rabies or lymphocytic choriomeningitis virus were completely inactivated by irradiation for 0.2 to 0.4 second; an exposure twice this length was necessary to inactivate lightly centrifuged 4 per cent suspensions of St. Louis encephalitis virus. Several lots of rabies vaccine inactivated by this irradiation technic consistently induced a higher degree of immunity in mice than did control phenolized vaccines. The irradiated rabies vaccine exhibited no significant loss of potency after six months storage at 5° C. Two lots of St. Louis encephalitis vaccine inactivated by the above technic developed a high degree of immunity in mice. Irradiation of rabies and St. Louis encephalitis viruses beyond the optimal time necessary for complete inactivation causes progressive diminution of antigenic potency. Several lots of completely inactivated poliomyelitis vaccine (the Lansing strain of virus) were prepared by the technic described. The virus was regularly inactivated in less than one second of exposure to the source of irradiation.

Such vaccines were proved to be completely inactivated by critical safety

tests in mice and monkeys. Mice immunized with two and especially three doses of the irradiated poliomyelitis vaccine developed significant resistance to intracerebral inoculation of the living virus, and neutralizing antibodies were found to be present in two to three weeks. Ten per cent suspensions of mouse brain infected with the virus of Japanese B encephalitis were inactivated in 0.5 second of exposure, and centrifuged 20 per cent chick embryo suspensions infected with the western and eastern types of equine encephalitis were inactivated in one and two seconds of irradiation, respectively. Allantoic fluid of embryonated eggs infected with influenza A and influenza B and concentrated by the red-cell absorption-elution technic was inactivated by irradiation for 0.2 second. Allantoic fluid infected with psittacosis and human pneumonitis viruses was inactivated in 0.1 second.

From the foregoing studies it is concluded that rapid inactivation with ultraviolet light can completely destroy even heavy suspensions of bacteria or viruses, and that in many instances the antigenic structure is not seriously denatured. Some of the irradiated vaccines appear to be definitely superior to heat-killed or chemically inactivated vaccines. In some instances the irradiated vaccines show potency, whereas the usual methods of inactivation appear to destroy the immunogenic capacity.

The future course of studies should be directed in three ways. First, the vaccines that appear to be potent on animal testing should be tested in man. Such a test is now under way with an irradiated polyvalent dysentery vaccine. Second, this method of producing vaccines should be extended to infectious diseases for which no satisfactory vaccine has yet been developed. This work should include infectious diseases in animals as well as in man. Third, if more potent antigens can be prepared with the technic of rapid ultraviolet inactivation, this may serve as a means for producing potent therapeutic antiserums for diseases against which there are no effective antiserums at the present time.

VENEREAL DISEASES

JOSEPH EARLE MOORE

FOR EXPERT ADVICE regarding the urgent problem of control and treatment of venereal disease, the armed services turned to the National Research Council and specifically to its Subcommittee on Venereal Diseases. As phases of this problem that required further research were identified, projects were sponsored by the Committee on Medical Research in various institutions with a view to advancing the knowledge of the prevention and treatment of these diseases.

There are five so-called venereal diseases usually transmitted by sexual contact: chancroid, lymphogranuloma venereum, granuloma inguinale, gonorrhea, and syphilis. Of these, the first three are less common in this country than syphilis and gonorrhea. On the other hand, in certain areas to which members of the armed forces were dispatched the other venereal diseases, particularly chancroid, are much more prevalent. In the light of this fact, it became imperative that proper measures for prophylaxis be developed against as many types of venereal disease as possible.

CHEMICAL PROPHYLAXIS OF VENEREAL DISEASE AND RELATED STUDIES

At the beginning of World War II the same system of chemical prophylaxis had been in use in the United States Army and Navy for about thirty years. This had been devised largely on clinical grounds. It consisted of initial urination (theoretically in order to wash out gonococci from the urethra), thorough washing with soap-and-water (theoretically to prevent chancroid and syphilis), the intraurethral injection of mild silver proteinate (theoretically to prevent gonorrhea), and the inunction of the parts with 33.3 per cent calomel ointment (theoretically to prevent syphilis). The adoption of these several steps in the old Army prophylactic system was based on scientific information only in respect of the use of calomel ointment for the prevention of syphilis. Earlier and not altogether satisfactory studies had suggested that soap-and-water was prophylactically effective against both chancroid and syphilis. The possible value of calomel ointment in the prevention of syphilis had been inferred from the original experiments of

Metchnikoff and Roux and from subsequent studies in experimental animals carried out by Nichols, Walker, Mahoney, and others. The use of silver proteinate for the prevention of gonorrhea rested on no other foundation than that it appeared to be of some value in the treatment of established gonococcal urethritis. Nothing was known of possible prophylactic agents against granuloma inguinale or lymphogranuloma venereum.

The estimate of the value of the whole prophylactic system rested entirely on poorly controlled and relatively unconvincing statistical studies carried out in the field. There was some reason to believe that if applied within one hour following exposure it might be of value, but this had not been definitely proved. A systematic study of prophylactic agents against the several venereal diseases was therefore carried out both in animals and in man.

CHANCROID

The prophylaxis of chancroid was investigated in two different clinics — at New York University and at the University of Virginia. Various prophylactic agents were tried in volunteers deliberately inoculated with chancroid. These studies showed that soap-and-water was of no value, but that sulfonamides locally applied in an ointment base or in vanishing detergents were effective in preventing the development of chancroidal infection. The ideal drug and concentration thereof appeared to be 15 per cent sulfathiazole, incorporated in an ointment base. There was some evidence that the combination of sulfathiazole and calomel was more effective against chancroid than sulfathiazole alone. These studies eventually led to the incorporation of 15 per cent sulfathiazole in the prophylactic ointment finally adopted by the armed forces.

LYMPHOGRANULOMA VENEREUM

Studies looking toward the possible development of a prophylactic agent against lymphogranuloma venereum were carried out primarily in the Squibb Institute for Medical Research. First it was shown that the virus could be propagated on the chorioallantoic membrane of the chick embryo. Efforts were made to adapt this experimental method to a study of prophylactic agents. Owing to the limitations of the method itself, these were not entirely successful. So far as could be determined, however, none of the drugs tested exercised any prophylactic effect against this disease. Efforts were made to produce experimental infection in animals comparable to that in man, with the hope that prophylactic agents could be tried out in vivo, but these attempts too were unsuccessful. As matters stand at the present time, there is no information available concerning the prophylaxis of lymphogranuloma venereum.

Granuloma Inguinale

The causative organism of granuloma inguinale had been successfully cultivated by Anderson of Vanderbilt University, again in the yolk of developing chick eggs. This discovery was confirmed by Rake of the Squibb Institute, working under an OSRD(CMR)[1] contract. However, efforts to establish experimental granuloma inguinale in animals or in human beings were unsuccessful, and there appeared to be no suitable method of testing prophylactic agents. As with lymphogranuloma venereum, therefore, no information indicating that this disease can be prevented by chemical prophylactic means has been developed.

Gonorrhea

At the beginning of these studies no satisfactory method existed of evaluating prophylactic agents against gonorrhea. Some hint of their value was gained by the local action of various compounds in established gonococcal urethritis. Studies of the local effect of the placing of sulfonamide ointment in the urethra of patients with already developed gonorrhea did not indicate that this method was likely to be effective in the prevention of the disease.

Efforts were therefore directed to establishing gonococcal infection in experimental animals, which had never previously been successfully accomplished. These took two directions. First, Justina Hill at the Johns Hopkins Hospital attempted to establish gonococcal infection on the vaginal mucosa of the immature female mouse. Second, C. Philip Miller, of the University of Chicago, undertook to establish gonococcal conjunctivitis or ophthalmia in the eye of the rabbit. Hill succeeded in establishing the temporary persistence of gonococci, if not actual infection. This was accomplished only after carefully testing a number of expedients calculated to lower the resistance of the host. None of these appeared to be successful in establishing genuine gonorrheal vaginitis, although with certain methods a high proportion of the animals showed persistence of viable gonococci for periods of forty-eight to seventy-two hours. Since actual gonococcal infection was apparently not established, the method did not lend itself to accurate study of chemical prophylactic agents. Nevertheless, Hill did survey the effect of a large number of prophylactic agents and demonstrated that, within the limitations of the method employed, 33 per cent calomel ointment was effective in destroying gonococci locally. Silver proteinate also gave promising results. Sulfonamides and a number of other salts of silver and mercury that were investigated seemed to be ineffective.

[1] This abbreviation is used throughout the book to designate contracts made by the Office of Scientific Research and Development on recommendation of the Committee on Medical Research.

Hill has published certain papers dealing with the development of her method (see Bibliography), and in addition has prepared at the request of the Committee on Medical Research two comprehensive surveys of the literature dealing with experimental gonococcal infection in animals and with experimental gonorrhea in man.

Miller, by means of the injection of virulent gonococci into the aqueous or vitreous of rabbits' eyes, finally developed a method that produced actual persisting infection for a period of many weeks in a high proportion of animals. An effort was made to study the effect of prophylactic agents introduced within the globe of the eye following such experimental infection. The results are scarcely applicable to the prevention of gonococcal urethritis but are particularly significant with regard to the chemotherapy of this infection.

Finally, confronted by what appeared to be the insuperable difficulty of producing gonococcal infection in experimental animals within the period of the war and in time for this to be of value to the armed forces, an experiment was organized to study the production of experimental gonorrhea in volunteers. This was carried out by Mahoney and his associates of the United States Public Health Service with the co-operation of the Federal Bureau of Prisons in the federal penitentiary at Terre Haute, Indiana. A team of investigators was sent to Terre Haute and somewhat over two hundred volunteers were employed. Efforts were made to produce experimental gonorrhea in these subjects by almost every conceivable expedient except the intraurethral inoculation of pus taken directly from the cervix or urethra of infected females or the natural method of infection — sexual intercourse. The former method was avoided because of the danger of transmitting syphilis simultaneously.

The results of this study (see Bibliography) indicated that it was not possible to produce experimental infection with any degree of regularity, either by the use of cultured gonococci of many strains or by direct patient-to-patient transfer of pus from natural or experimental gonorrhea. Only about half the volunteers became infected. It seems probable that this duplicates the epidemiologic situation in nature. There is reason to believe that, for reasons not clearly defined, not all men exposed to an infected woman will become infected.

Since experimental gonorrhea could not be produced with any degree of regularity in volunteers, this method likewise did not lend itself to a study of chemical prophylactic agents. The study did, however, demonstrate that resistance to sulfonamides was an inherent property of the strain of gonococci employed. When volunteers were infected with a strain of organism previously known to have been sulfonamide-resistant in vivo, with such resistance further demonstrated in vitro, all the infections produced were likewise sulfonamide-resistant, requiring penicillin for cure. On the other
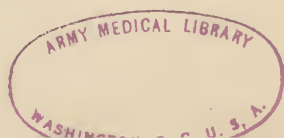
hand, when experimental infection was produced with a sulfonamide-susceptible strain, it could always be readily cured with sulfathiazole.

The net result of these experiments in chemical prophylaxis against gonorrhea was nil so far as any practical application during the period of the war was concerned. It is still unknown whether any prophylactic agent, including the silver proteinate that the armed forces have used for thirty-five years, has any value in the prevention of this disease.

SYPHILIS

The primary need for study in the chemical prophylaxis of syphilis was to define more clearly the effect of calomel. Investigations looking to this end were carried out in a number of different laboratories, particularly those of Chesney and Eagle at Johns Hopkins and of Fleming at the University of North Carolina. It was shown that in the dosage used in man, 4 gm. of a 33 per cent calomel ointment, corresponding roughly to 200 mg. of calomel per kilogram of body weight, the preparation did have some effect in preventing experimental infection in rabbits. It was demonstrated that the efficacy of calomel was dependent in part on particle size. So-called "micronized" calomel ointment, with a particle size of 1 micron or less, was much more effective than the cruder preparation previously employed by the Army, which contained particles up to 100 micra in diameter.

The results were also considerably influenced by the ointment employed. The problem of ointments was investigated in a number of different laboratories, particularly those of the Food and Drug Administration, the Warner Institute for Medical Research, the Squibb Institute for Medical Research, and that of Chesney and Eagle. A large number of ointments — three hundred or more — were studied in experimental animals with particular attention to the absorption of drugs contained therein and to such factors as primary irritation of skin and mucous membranes. The results stimulated the further trial of a new ointment combining 33 per cent calomel with 15 per cent sulfathiazole. This ointment, in a vanishing-cream base, was pharmaceutically much more acceptable for general use than the greasy lanolin ointments previously employed. The studies of the prophylactic effect of calomel in experimental animals likewise confirmed the original impression of Mahoney that calomel ointment exerted not only a local but also a systemic effect in prophylaxis.

Since the efficacy of calomel ointment in the prophylaxis of syphilis could not, however, be established with absolute certainty, other studies were undertaken by Eagle and Mahoney on the effectiveness of arsenic applied in solution or in ointment bases. These studies indicated that phenyl arsenoxides were much more effective than calomel in the prevention of experimental

syphilis in animals and that their action was purely local rather than systemic. It was likewise demonstrated that in the concentrations employed in experimental animals these arsenical drugs were not irritating or apparently sensitizing in man. Unfortunately, the experimental information became available so late in the war that opportunity for extensive field testing of arsenic-containing preparations was not possible. Such a trial may be carried out in further experiments by the United States Public Health Service.

SUMMARY OF RESULTS

From the theoretical standpoint, the results of these investigations were in a sense disappointing. The major achievements were the demonstrations that soap-and-water was not effective against chancroid but that sulfathiazole in ointment would protect against it, and that arsenic was superior to calomel in the prevention of syphilis in experimental animals. No agents effective in preventing granuloma inguinale or lymphogranuloma venereum were developed. Likewise no information was provided as to the effectiveness of any substance in the prevention of gonorrhea.

From the practical point of view, however, the information outlined above did permit the adoption by the Army of a new single-tube "Prokit" containing 15 per cent sulfathiazole and 30 per cent calomel in a vanishing-cream base. This was easier of application than station prophylaxis or the two-tube prophylactic kit previously employed. Such inconclusive evidence as is available from field trials suggests that the single-tube "Prokit" is fully as effective as station prophylaxis in the prevention of venereal disease. The demonstration of the usefulness of penicillin in the treatment of gonorrhea is described elsewhere. It is worthy of emphasis here that the availability of this antibiotic agent with a rapid curative effect in this disease, and without the hazard of toxic side effects, was responsible for the prompt restoration to active service of thousands of men.

THE TREATMENT OF SYPHILIS

By far the most important problem faced by the Army in the field of venereal disease was the selection of adequate and safe methods for the treatment of syphilis and the organization of their administration. In the early acute phases the patient is not ill or disabled. The principal purposes of treatment are to prevent him from infecting others and to prevent the late disabling manifestations of the disease.

From 1492 to 1943 progress in the treatment of syphilis had been slow and hesitating and the results incomplete and inconclusive. Until 1909 all treatment of this disease was empirical, and the only really useful informa-

tion that emerged concerned the efficacy of mercury in some cases. This had survived as the single important ingredient in the medieval treacles and other compounds administered over a period of four hundred years.

Arsenic in its various forms, beginning with Ehrlich's discovery of "606" in 1909, had been administered by physicians more or less by trial and error. With a few outstanding exceptions, no successful attempts had been made to follow cases for long periods after treatment, and no series of cases of adequate size had been studied sufficiently to yield definitive information. It is to be emphasized that to measure the influence of any therapeutic procedure on the course or outcome of syphilis requires that the case be observed for months or even years. Unlike such diseases as pneumonia, in which the brilliant results of chemotherapy are apparent within a few hours or at most a few days, syphilis is a chronic disease. A patient apparently cured today may for unpredictable reasons suffer a relapse months or years later.

The Subcommittee on Venereal Diseases of the National Research Council assembled the available information regarding the various methods suitable for the treatment of syphilis under military circumstances. They recommended that infected persons undergo a course of treatment lasting for twenty-six weeks and composed of forty injections of Mapharsen and sixteen of bismuth. All forms of arsenic treatment of syphilis have a significant mortality rate, owing to the poisonous effects of this chemical. In the opinion of the Subcommittee, no reasonably safe system of arsenic treatment had been evolved that could be administered in less than twenty-six weeks. Attempts to administer therapeutically adequate quantities of arsenic within a few days had been accompanied by a prohibitive percentage of complications, some of them fatal.

Under OSRD(CMR) contracts attempts to discover more efficacious and less toxic compounds of arsenic were continued. Other investigators without such contracts but in close collaboration co-operated with the Subcommittee on Venereal Diseases and the Committee on Medical Research in an extensive study of intensive arsenotherapy in experimental syphilis of animals. From these investigations there emerged a number of significant facts. First, the toxic dose of arsenic, as exemplified by Mapharsen and its analogues, was directly related to the time period of its administration: the shorter the interval within which a given amount of treatment was compressed, the higher was the mortality rate in the experimental animals treated. Second, and conversely, the curative dose of arsenic, again as exemplified by Mapharsen and its analogues, was approximately the same within the limitations of the experiments, regardless of the total period of administration. Whether the drug was administered by means of a single massive intravenous injection, by intravenous drip over a period of several days, or by interrupted injections at various time intervals, extending up to a total treatment period of six

weeks, the curative dose in experimental syphilis in rabbits was about 7 mg. per kilogram of body weight.

These two facts made it evident that there was no optimum method of treatment of early syphilis with arsenic. If it was considered desirable to administer a total dose of, for example, 7 mg. per kilogram, the time period over which this dose was administered determined the safety of the treatment. With these facts in hand it became possible to predict mortality rates in rabbits that were translatable with extraordinary fidelity to human beings. In both rabbits and man it became clear that the administration of a curative dose of arsenic within a period of ten days or less yielded a mortality rate of approximately 1 in 200. Most, if not all, of the deaths in man occurred because of toxic encephalopathy, the incidence of which was about double the death rate. The mortality rate could be decreased to any desired level, and practically to the point of disappearance, by spacing individual treatments in such a manner as to prolong the total period of administration.

These considerations influenced the Subcommittee on Venereal Diseases to advise the armed forces against the use of intensive arsenotherapy by means of the so-called "five-day" treatment or intensive modifications thereof, and this form of treatment was not utilized in the military services in this country. In combat areas, on the other hand, and particularly in the European Theater of Operations, a modification of intensive arsenotherapy was developed requiring twenty days for completion. This was carried out in a substantial number of patients with a comparatively low risk.

The combined experimental studies in rabbits and clinical studies in man likewise demonstrated a third significant point: that the curative dose of arsenic in man was approximately three to five times that in the rabbit. Whereas the latter dose was approximately 7 mg. per kilogram of body weight, that in man ranged between 20 and 35 mg., requiring the administration of a minimum of 1200 mg. of Mapharsen (or an analogue) to the average 60-kilogram man with early syphilis.

A fourth point of major importance was developed both in experimental and in clinical studies; namely, that the addition of bismuth to arsenic exercised an apparently synergistic effect and improved the results obtainable with either drug alone.

An additional point of interest, although not of major practical import, was brought out by studies in the experimental laboratory. It was here demonstrated that when arsenic (Mapharsen) was administered to animals during induced fever, its toxicity was approximately doubled but its therapeutic efficacy was increased about fourfold. As a corollary to this study another was carried out in which distribution of arsenic in the tissues was determined following its administration at normal body temperatures and at fever temperatures.

The twenty-six-week treatment system was employed by the armed forces

until the middle of 1944, when it was replaced by treatment with penicillin. A preliminary analysis of the results of this system has recently been made in a published report by Lieutenant Colonel Thomas Sternberg and Major William Leifer. This study of 3000 cases indicates that the twenty-six-week treatment system was extremely satisfactory, producing an over-all failure rate in early syphilis in the Army of approximately 5 per cent. The mortality rate was roughly 1 in 30,000. It is estimated that from 200,000 to 300,000 men were treated in the Army and Navy by this system, with results superior to those previously attained by any treatment system in civilian life.

The Treatment of Arsenic Intoxication with BAL (British Anti-Lewisite)

In collaboration with the Committee on War Gases of the National Research Council, the Subcommittee on Venereal Diseases fostered experimental and clinical studies in the use of BAL (2-3-dimercaptopropanol) in arsenic poisoning. It was demonstrated that experimental animals could be protected against lethal doses of arsenic by the administration of BAL simultaneously with or shortly after treatment. It was also shown that the administration of BAL enormously increased the urinary output of arsenic when applied in the treatment of arsenic poisoning in human beings. It has likewise been shown with reasonable conclusiveness that BAL is of value in the treatment of arsenical toxic encephalopathy and of post-arsenical dermatitis. The mortality rate from arsenical toxic encephalopathy, which without the use of BAL appears to be 50 per cent or greater, can probably be reduced to 20 to 25 per cent by the use of this product. In a substantial number of patients with post-arsenical dermatitis treated with the drug, either by ointment or parenterally, there were no deaths, and the duration of illness seemed to be materially shortened.

As a result of these studies, BAL was distributed to large syphilis treatment centers, both in the armed forces and in civilian communities in the United States.

Effect of Penicillin

In June 1943, experimenters at the United States Marine Hospital on Staten Island demonstrated in experimentally infected rabbits and in four human beings that penicillin exercised a prompt and powerful effect on the responsible organism of syphilis, bringing about the destruction of treponemes in open lesions, with consequent healing, and reversal of positive serologic reactions. In view of the fact that penicillin is almost completely innocuous to man, even in enormous doses, this was a valuable discovery.

At this time, owing to the shortage of penicillin and the urgent and ex-

traordinary need for it for the treatment of battle casualties and serious acute infections in the armed forces, it was decided that the available supply of the drug should be devoted not to a large-scale human experiment but to the further study in the experimental laboratories of Mahoney and Eagle of the effect of penicillin in rabbit syphilis. Within three months, however, the supply of penicillin had greatly increased, and the results from these laboratories were so encouraging that in September and October 1943, steps were taken to arrange for large-scale human trial. The co-operation of the Army, Navy, and United States Public Health Service was promptly enlisted to set up experimental treatment centers in various installations of these services. In addition, over twenty-five civilian institutions engaged in the study under OSRD(CMR) contracts.

The pressing need of the armed forces was for information regarding the effect of penicillin in early syphilis, and secondarily for knowledge regarding its usefulness in latent syphilis and neurosyphilis. It was nevertheless decided that while the major efforts should be centered on these phases of the infection, the investigation should not neglect to explore the value of penicillin in the treatment of other stages of the disease.

There was organized under the Subcommittee on Venereal Diseases what was called the Penicillin Panel, which included representatives of the Army, Navy, United States Public Health Service, Committee on Medical Research, Subcommittee on Venereal Diseases, and Committee on Chemotherapeutic and Other Agents. In this panel the design of the experiment was discussed and developed and from time to time evaluated.

It was evident that so far as early syphilis was concerned, there was presented an unparalleled opportunity for quantitative study of the effect of penicillin. It was arranged that all co-operating institutions, governmental or civilian, should select their patients on a uniform basis, examine the cases, and record information concerning the history and physical findings in a uniform manner. They were to apply treatment schemes prescribed by the Penicillin Panel, follow the patients so far as possible in an identical fashion, and record the results of all these observations on specially designed forms, which were to be forwarded to the Central Statistical Unit for analysis. This unit was established in the Department of Biostatistics of the Johns Hopkins School of Hygiene and Public Health.

The original effort was to evaluate the time-dose relationship that had been used in the first four patients treated by Mahoney. The method of administration of penicillin was to give it in aqueous solution by the intramuscular route, with a three-hour interval between injections. The total dose arbitrarily chosen (without previous experience or any guidance from the experimental laboratory) was 1,200,000 units, and the duration of treatment (again arbitrarily chosen) was set at seven and a half days. The method of administration and the interval between injections were based on

pharmacologic evidence that had by this time accumulated in the general use of penicillin. It was decided at the beginning to hold these factors constant, and to determine the relative effects of total dosage within the range of 60,000 to 9,600,000 units and those of total time of administration within the range of four to thirty-two days. The dosage steps were as follows: 60,-000, 300,000, 600,000, 1,200,000, 2,400,000, 4,800,000, and 9,600,000 units. The total duration of treatment was increased in the same manner; that is, four, eight, sixteen, and thirty-two days, with variation as to dose in each time interval. Likewise to be explored in human beings were the combination of penicillin with other chemotherapeutic agents — for example, arsenic, bismuth, and fever therapy — and a study of absorption-delaying methods of administration of the drug.

Simultaneously with these clinical studies in early syphilis, a group of six co-operating laboratories of experimental syphilis was organized under the direction of a subcommittee of the Penicillin Panel. These laboratories were to study the optimum method of administration of penicillin in early and late syphilis of rabbits.

The experimental and clinical studies in early syphilis yielded valuable information. It was demonstrated in experimental animals that the CD_{50}[2] of commercial penicillin administered in twenty to twenty-four injections every four hours day and night for a four-day period was 1500 to 2000 units per kilogram of body weight, and that the CD_{95} was about 3000 units per kilogram. It was further shown that the curative dose of penicillin was a function of the combination of total dose, duration of treatment, and frequency of injection. When penicillin was combined with arsenic in treating rabbit syphilis, there was a synergistic effect, so that the curative dose of each drug when the two were administered simultaneously was approximately one fourth the curative dose when either one was given separately. It was also shown that penicillin in experimental syphilis was more effective at fever temperature than at normal body temperature.

In the human experiments it became obvious that, within the time periods assigned for total duration of treatment — that is, from four to eight days — any total dosage less than 1,200,000 units was relatively ineffective, and that a total dosage of 1,200,000 units administered within either four or eight days produced an apparent-cure rate in early syphilis approximating 80 per cent. By the spring of 1944 this information was considered sufficiently satisfactory to justify a recommendation by the Subcommittee on Venereal Diseases on April 20 that penicillin be substituted for arsenic and bismuth in the treatment of syphilis in combat areas, and on June 29 it was recommended to the Army and Navy that this drug be adopted throughout both services in all theaters of operation for the treatment of early and latent syphilis. On the basis of information then available, the treatment plan

[2] The amount necessary to cure 50 per cent of infected animals.

advised for both early and latent syphilis was 2,400,000 units of penicillin administered by the intramuscular route in divided doses every three hours day and night, to a total of sixty injections in seven and a half days. This plan was adopted by the armed forces at a slightly later date, and from that time until April 1946, over 250,000 patients in the Army and Navy with early and latent syphilis were so treated.

In the meanwhile, data concerning the treatment of early syphilis in human beings have continued to accumulate in the Central Statistical Unit, so that at the present writing records of some 20,000 cases are available for study. These cases include patients treated by about thirty different treatment schedules — some with penicillin alone, others with penicillin and arsenic, others with penicillin and bismuth, others with penicillin and fever, and a substantial new group treated with penicillin by an absorption-delaying method, the drug being suspended in a mixture of peanut oil and beeswax.

It has become obvious from the human study that any kind of treatment scheme of early syphilis requires a minimum of two years for evaluation. For this reason it is not possible at present to make definitive statements with regard to any total penicillin dosage larger than 2,400,000 units or to compare the periods of total duration of treatment beyond the limits of four to fifteen days. Within these limits, however, it has been shown that the results are apparently equally satisfactory whether the total duration of treatment is four, eight, or fifteen days. The results improve progressively with increased dosage, but the magnitude of difference in percentage of favorable outcome tends to diminish as dosage is stepped up. With 2,400,000 units of penicillin administered in eight days, the system employed by the armed forces, the failure rate within the first year is approximately 15 per cent. Failure or success cannot be estimated by original effect in terms of disappearance of treponemes, healing of lesions, or serologic reversal, since the results are essentially identical in respect of these factors, regardless of total dose. Failure can only be measured by the incidence of relapse, either clinical or serologic (including reinfection), and by seroresistance at the end of one year after treatment. It is the time required for accumulation of such data that makes it necessary for a given treatment system to have been followed for a minimum of twelve to twenty-four months before its results can be determined.

The experiments in the treatment of syphilis in human beings likewise indicate that the administration of arsenic with penicillin improves the results as compared with penicillin alone; the same is true of penicillin and bismuth. It is not yet clear whether penicillin and fever is better than penicillin alone, although the present indication is that this combination is not effective in man. Sufficient time has not yet elapsed to permit any conclusion with respect to the value of absorption-delaying methods of administration of penicillin in the treatment of syphilis.

While a 15 per cent failure rate from 2,400,000 units of penicillin in eight

days is considerably greater than the failure rate under the best available methods of treatment with arsenic and bismuth (metal chemotherapy), which is in the neighborhood of 3 to 5 per cent, the advantage is still with penicillin. With the latter drug all patients can be treated, whereas with arsenic and bismuth, which for safety require a minimum of twelve and a maximum of twenty-six weeks of treatment, a substantial proportion of patients either do not complete the required course or take treatment irregularly over a much longer period of time. To the extent to which arsenic and bismuth are not given by a regular schedule, or a total course of treatment is completed only in part, the results are substantially less good than the 3 to 5 per cent failure rate mentioned above. Moreover, penicillin has the inestimable advantage of freedom from toxicity. No deaths from its use have been reported, whereas metal chemotherapy by any system carries with it a mortality rate that, although of course capable of adjustment, cannot be reduced below a probable 1 in 30,000 patients treated.

In other forms of syphilis the results of penicillin therapy, as determined from the nationwide study, may be briefly outlined as follows.

Prevention of Prenatal Syphilis in Infants

The most successful application of penicillin in the treatment of syphilis has been in preventing the development of congenital syphilis by treatment of infected pregnant mothers. Over 500 pregnant women with early syphilis have now undergone treatment with this drug. More than 95 per cent of the infants have been born alive and free from infection. In the remaining relatively small number of failures, the reason for failure is usually readily determinable, and it seems entirely possible that by recognition of such reasons and the application of re-treatment during pregnancy when necessary congenital syphilis may eventually be completely eliminated with entire safety to the mother.

Infantile Congenital Syphilis

Penicillin has been used in the treatment of 250 to 300 infants born with congenital syphilis. The dosage scales have been roughly comparable to those used in adults and have been chosen on the basis of an appropriate number of units per kilogram of body weight. At present the recommended total dose of penicillin for infants is between 100,000 and 400,000 units per kilogram, administered over a time period of eight to fifteen days. The results have been fully as satisfactory as those in adults, and in some respects more so. The mortality rate, always high in infantile congenital syphilis, is certainly no higher than with earlier methods of treatment and is not ascribable to the treatment itself. There is a lower incidence of clinical relapse in penicillin-treated infants than in adults, and serologic reversal now appears to be equally frequent.

Latent Syphilis

Although a large number of patients with latent syphilis in the armed forces have been treated with penicillin, no attention has been paid to this problem in civilian clinics, and no definite statements regarding the effect of the drug can be made. The results of any form of treatment in this stage of the infection are measurable only by serologic response on the one hand or by eventual clinical outcome on the other. Before the advent of penicillin it was already clear that these two factors bore no necessary relation to each other. Serologic response in latent syphilis, treated by whatever method, is largely a function of the duration of infection. If the latent infection is recognized within the first few months or even the first two years of its inception, serologic reversal is accomplished in almost the same time as in manifest early syphilis. On the other hand, if the infection has existed for as long as two to four years before treatment, seroresistance is the rule. This is as true of penicillin-treated cases as of those receiving metal chemotherapy. In late syphilis in general, whether latent or with outspoken lesions involving various structures of the body, penicillin is no more effective than arsenic and bismuth in causing reversal of serologic reaction.

The aim of treatment in latent syphilis therefore becomes entirely that of preventing development of the disease. To determine whether this aim has been accomplished requires prolonged observation of a large series of patients for at least twenty-five to thirty years. Since penicillin has been in use in the treatment of syphilis for slightly less than three years, nothing whatever can be said of its effectiveness in this stage of the disease. Its use in latent syphilis is justified only by analogy and on the basis of the profound demonstrable effect in patients with outspoken evidence of syphilitic disease, whether early or late.

Benign Late (Gummatous) Syphilis of the Skin, Mucous Membranes, and Bone

A moderate number of patients with gummatous lesions involving these nonvital structures have been treated with penicillin. The most that can be said at the moment is that this drug exercises a healing effect on such lesions equal or superior to that of arsenic. The eventual outcome, measured in terms of subsequent relapse, will not be determinable for a number of years.

Cardiovascular Syphilis

What has been said of latent and benign late syphilis applies even more to cardiovascular syphilis. Determination of the efficacy of any therapeusis requires the treatment of a large number of patients who are not subsequently re-treated if the original treatment effort seems to have been unsuccessful and who are followed from treatment until death. The efficacy

of treatment is measured only partly by relief of symptoms but principally by prolongation of life expectancy beyond that of untreated patients. For these reasons an organized study of the effect of penicillin in this stage of the infection has not yet been undertaken. A few patients have been treated, enough to indicate that therapeutic shock (the Jarisch-Herxheimer reaction [3]) from penicillin may prove to be a matter of major importance. Certainly the experience of the past suggests that powerful treponemicidal drugs should be given cautiously in this group of patients. Preliminary information indicates that the initial doses of penicillin should not exceed 1000 units and should be increased gradually, probably over a five-day period, to full therapeutic doses.

Interstitial Keratitis

This distressing ocular lesion of late congenital syphilis responds to penicillin only in an irregular and unpredictable fashion, and to about the same extent as has been previously observed with other methods of treatment.

Neurosyphilis

One of the outstanding achievements of penicillin has been to reduce the incidence of asymptomatic neurosyphilis among patients treated in the early stages of syphilis. When untreated, from 15 to 40 per cent of such patients may be expected to develop spinal-fluid abnormalities within the first two years; with penicillin treatment this percentage is reduced to 2 per cent or less.

A large number of patients with various types of neurosyphilis have been treated with penicillin. These types have ranged from the asymptomatic variety, in which the diagnosis hinges solely on routine examination of the spinal fluid, up to and including the most serious manifestations of the disease; that is, tabes dorsalis and general paralysis of the insane. It has been shown that the drug when administered either intravenously or intramuscularly exerts a profound effect, in spite of the fact that it does not apparently penetrate into the nervous tissue or into the spinal fluid in appreciable concentration.

[3] This is the only important reaction occurring after the use of penicillin in syphilis. It is apparent in all stages of syphilitic infection. It is usually manifested by fever appearing within the first twenty-four hours of treatment, ranging in height from 99.5° to 105° F., persisting for a few hours, and then disappearing. In patients with obvious lesions, as, for example, in early syphilis, this febrile reaction is frequently associated with a temporary intensification of the lesions previously present. The reaction occurs in about 75 per cent of all patients with early syphilis and in a substantially smaller proportion of those with late syphilis. It has no clinical significance except in cardiovascular syphilis, where, theoretically at least, it may produce serious damage, and in certain forms of neurosyphilis, especially paresis, where convulsive seizures, vascular accidents, and so forth have apparently been precipitated by the incautious use of initial large doses of the drug. Here it seems desirable to avoid it. This can usually be accomplished by the administration of small doses for a day or two.

The favorable effects of penicillin treatment of neurosyphilis are most obviously reflected in the changes in the spinal fluid. Without exception, elevated cell counts and protein content return promptly to normal, sometimes within the actual period of treatment, and in practically all cases within a month thereafter. Other spinal-fluid abnormalities, including a positive Wassermann reaction and an abnormal colloidal-gold curve, respond more slowly, but there is a continuing favorable effect that has persisted for at least the present duration of the experiment, about two years. This favorable effect occurs in all types of neurosyphilis.

From the clinical point of view, it is more difficult to evaluate the effect of the drug because of the extraordinarily protean character of neurosyphilis. On the whole, however, it appears that penicillin is more efficacious in the treatment of any type of neurosyphilis than chemotherapy with arsenic and bismuth. It appears to be less effective than fever therapy with induced tertian malaria. There has, however, been an accumulation of evidence that the combination of fever therapy with simultaneously administered penicillin may be more effective than either treatment method alone. It is fortunate in this respect that penicillin exercises no therapeutic effect on malaria, thereby permitting its continued administration during malarial therapy. The information so far gathered permits the hope that the treatment of any type of neurosyphilis may be completed with the combination of fever and penicillin therapy within four to six weeks, thereby obviating the prolonged additional period of chemotherapy that has been customary in the past. The evidence in this respect has been sufficiently convincing so that the Army and Navy have adopted the routine of fever and penicillin treatment in their various neurosyphilis centers.

THE CHANGING CHARACTER OF PENICILLIN

Information concerning the chemical nature of penicillin itself and of the several substances composing commercial penicillin was withheld during the war. Nevertheless, information began to accumulate by personal communication from a variety of sources to the effect that penicillin, as commercially supplied, is composed of at least four different penicillin species: G, X, F, and K. These species differ from each other chemically only in the side-chain structure attached to the basic nuclear molecule. They also differ from each other in their potency in vitro against *Staphylococcus aureus,* penicillin G and F having a potency of 1667 u./mg., X a potency of 900–950 u./mg., and K a potency of 2300 u./mg.

There were increasing indications that penicillin as commercially supplied by different manufacturers contained varying amounts of these several species and, indeed, that the amounts might vary in the product of the same manufacturer from time to time. Eventually it became clear that penicillin

as originally supplied in 1943 and early in 1944 contained predominantly the species G or a mixture of G and F. At some time during 1944 the drug industry generally substituted a strain of *Penicillium chrysogenum* for *P. notatum*. This, together with various modifications adopted in the culture of the mold and in the purification of the final product, resulted in the appearance in commercial penicillin of substantial amounts of penicillin K.

Commercial penicillin has also during the nearly three years' conduct of the syphilis experiment changed markedly in another respect; that is, in increasing potency against *Staph. aureus* in vitro, in terms of units per milligram. As originally supplied, the potency of commercial penicillin was about 200 u./mg., whereas at present it is 900 to 1400 u./mg. This purification has resulted in a decrease in the amount of impurities present. As regards these impurities certain suggestions had begun to appear in the literature to the effect that penicillin of low potency and therefore containing a high proportion of impurities might possibly be more effective in various respects than more highly purified or crystalline forms. Leads in this direction were obtained in experimental syphilis in rabbits, in the action of crude and purified penicillin against sarcoma cells in tissue culture, and in the similar action of such substances against streptococcal infections in mice and against the development of sea-urchin eggs in vitro.

Early in 1945 the Penicillin Panel of the Subcommittee on Venereal Diseases, recognizing that experimental studies of penicillin species G, X, F, and K and of the impurities present in commercial penicillin should be carried out, made efforts, without success, to obtain crystalline penicillin in preparations containing a high proportion of impurities. Eventually, arrangements were made with pharmaceutical houses for supplies of penicillin species, and small quantities were obtained of crystalline G, X, and K and slightly larger quantities of F and K, each of the latter being stated to be 90 per cent pure. These species were distributed among six laboratories of experimental syphilis, which undertook to examine them on a co-operative basis.

About the middle of February 1946, it was reported from two of these laboratories that penicillin K was ineffective in syphilis of the rabbit in the largest dose employed; that is, 16,000 u./kg. Simultaneously it was shown that G was fully as effective as commercial penicillin, as supplied early in 1944. The CD_{50} of penicillin G and of this earlier commercial penicillin was in the order of 1500 to 2000 u./kg. It therefore appeared that penicillin G was at least ten times as effective as penicillin K in experimental syphilis of animals. Information regarding the relative efficacy of penicillins X and F in this disease is not yet available. Almost simultaneously with the demonstrated inefficacy of penicillin K, information developed that in human beings commercial penicillin, as supplied since mid-1944, was not as effec-

tive against syphilis as the products supplied before that date. These two facts seemed almost certainly to be related, and prompted a further exploration of penicillin K as contrasted with other penicillin fractions.

It has been shown in several laboratories that the inefficacy of penicillin K is probably not due to any lack of intrinsic bactericidal power, but instead depends on the pharmacologic fact that, unlike penicillins G, X, and F, this fraction is in large part rapidly destroyed in the animal or human body. It has also been shown that penicillin K is from one seventeenth to one sixth as effective as penicillin G in streptococcal and pneumococcal infections of mice.

BIOLOGIC FALSE-POSITIVE SEROLOGIC TESTS

Before and during the war, mass blood testing was adopted by industry, in hospitals, and by law in premarital and prenatal examinations. Later the Selective Service performed blood tests on all draftees. These conclusively demonstrated an unexpected number of biologic false-positive serologic reactions for syphilis, caused by a variety of different conditions. For example, in malaria such a reaction appears at some time during the course of the acute illness in 100 per cent of cases. Following vaccination for smallpox the incidence is about 20 per cent, and this same incidence prevails during or after a number of acute infections including infectious mononucleosis, atypical virus pneumonia, mumps, acute upper-respiratory conditions, including the common cold, and various other infectious diseases particularly prevalent in the armed forces.

Information began to accumulate indicating that most normal human beings possess in their blood serum a tiny quantity of reagin or a reagin-like substance, demonstrable by various special technics but so small in amount as under normal circumstances not to interfere with serodiagnostic tests for syphilis. It was likewise indicated, however, that about 20 per cent of the normal population were probably potential reactors in the sense that under the influence of an extraneous stimulation, such as the virus infections mentioned above, this substance, normally present in infinitesimally small quantities, might increase in quantity to such a point as to interfere with standard serodiagnostic tests and to produce biologic false-positive reactions. It was likewise suggested by clinical evidence arising from American Red Cross blood-donor centers that the mere donation of a large quantity of blood on one or several occasions might produce a temporary biologic false-positive reaction.

The frequency with which such reactions occur and the high incidence of extraneous stimulating factors, both in the civilian population and within the armed forces, made it desirable to conduct a series of investigations on fundamental factors related to the production of these reactions and their

differentiation from the positive blood reactions characteristic of syphilis. As one step in this investigation an effort was made in a prison population deliberately to produce biologic false-positive reactions by repeated blood donations. This experiment, carried out on a small scale, gave negative results. It did not, however, disprove the possibility that blood donation might in a very small proportion of cases be an exciting factor.

Another investigation was devoted to a study of the serologic pattern produced in various stages of syphilitic infection and in patients with known or suspected false-positive blood reactions. In addition, efforts were made to evaluate two so-called "verification tests," especially that proposed by Kahn. This study demonstrated that there was no constant serologic pattern that could be utilized to differentiate true from false positive reactions, and that the verification tests, including that of Kahn, were of no value. It was shown that the verification phenomenon formerly assumed to prove or disprove the fact of syphilis depends instead on the reagin titer of the serum in question. In patients with high-titer serums, whether due to syphilis or to some other cause, the Kahn verification test gives results of the so-called "syphilitic type." Conversely, with low-titer serums, again whether due to syphilis or to some other cause, this test usually provides results of the so-called "biologic type."

An extensive physicochemical study of the phenomenon of biologic false-positive reactions has been carried out by Neurath at Duke University. He has shown that reagin produced by syphilis is associated with a G–2 fraction of globulin. He has discovered several characteristics that promise to lend themselves to serologic differentiation between true and false positive reactions. The most important of these is that the serologically active euglobulin fraction prepared either electrophoretically or chemically from false-positive serums has a higher titer than the whole serum, whereas with serums from patients with syphilis the titer of this fraction is comparable to or less than that of the whole serum. Second, it has been shown that the addition to the isolated globulin fraction of a heat-stable serum inhibitor prepared from the crude serum-albumin fraction causes complete inhibition of the former's activity in the case of false-positive reactions, whereas with serums from patients with syphilis only partial inhibition, or none at all, is observed.

With these criteria applied to a large number of serums from patients with proved syphilis, proved false-positive reactions, and normal serums, it has been shown that 90 to 99 per cent of all serums from patients with syphilis give a syphilitic type of reaction, whereas 90 to 99 per cent of all false-positive reactors give a characteristic differential reaction. Studies are now in progress under other auspices to determine the nature of the inhibitor, its source, and the mechanism of inhibition by serum albumin.

TROPICAL DISEASES

LUCILLE R. FARQUHAR

A CONFERENCE was arranged in July 1943, at the request of the Surgeons General of the Army and Navy and the United States Public Health Service, to discuss the medical problems that might be expected to develop following contact by members of the armed forces with certain tropical diseases endemic in the Pacific, African, and Asiatic theaters of war. The Navy had already encountered filariasis in the Samoan Islands. It was anticipated that amebiasis, schistosomiasis, and leishmaniasis would also be encountered and might, like malaria, account for considerable wastage of troop strength by serious or disabling disease. No drug had been found that was successful in the treatment of filariasis, and the standard remedies for the other three diseases left much to be desired. The conference recommended that competent investigators be procured to study new chemotherapeutic agents of possible value against these diseases. In the interest of the public health it was further recommended that an effort be made to discover whether suitable intermediate hosts for filariasis, schistosomiasis, and leishmaniasis existed in this country.

In large measure the problem of new intermediate hosts was left to the United States Public Health Service, which had already investigated the small focus of filariasis in Charleston, South Carolina, and had demonstrated the mosquito vector there. Provision was made, however, by OSRD(CMR) contracts to investigate as possible transmitters of leishmaniasis certain flies of the genus Phlebotomus known to exist in the Rio Grande Valley of Texas, and to survey native fresh-water snails that might act as intermediate hosts of schistosomes. Before and during the African campaign considerable emphasis was placed on possible hosts of *Schistosoma mansoni* and *S. haematobium;* later, the search was intensified for snail species that might permit development of *S. japonicum.* The United States Public Health Service surveyed the southern and western United States, and investigators of the Office of Scientific Research and Development searched for possible snail hosts of *S. japonicum* in the northeastern states.

The primary effort of the group of scientists was, however, to investigate new chemotherapeutic agents. When the group was chosen initially, the talents of pharmacologists well versed in the newer agents developed in

other fields, notably the sulfonamides, as well as the knowledge of parasitologists and physicians familiar with the diseases in question, were considered indispensable. The urgency presented by numerous cases of filariasis in certain units of the Navy led initially to a more intensive effort in that field; later, for similar reasons the emphasis was shifted to intensive study of schistosomiasis and amebiasis.

It soon became apparent that the work would be enormously facilitated if a central agency collected new chemicals for screening and circulated the results and pertinent information within the group. Accordingly, the Divisions of Medical Science and Chemistry of the National Research Council set up the Chemotherapy Center for this purpose in 1944. Compounds were solicited by this bureau from academic and commercial laboratories. Some thirteen hundred chemicals were received, the bulk of them having been supplied gratis by commercial drug houses. The Chemotherapy Center also served to knit the small group of investigators and chemists into a closely co-operative unit.

FILARIASIS

Human filariasis is a disease due to the implantation, by the bite of an infected mosquito, of microscopic wormlike organisms, which collect in the lymph nodes and obstruct the flow of lymph from the corresponding part. This lasting obstruction causes the part to swell and produces the dreaded deforming complication known as elephantiasis.

At the outset a considerable volume of effort was wasted in parallel in vitro and in vivo screening tests. Both the microfilariae and the adult filariae were used for the in vitro studies. It became apparent that unless the comparative activity of a series of related compounds was studied under rigidly controlled conditions, tests in vitro would be meaningless, and this method was largely abandoned in favor of screening in vivo in the naturally infected cotton rat. The pleural cavity of such animals harbors a filariid worm, *Litomosoides carinii*. For supplemental studies dogs infested with heartworms were used. *L. carinii* is believed to be more closely related to *Wuchereria bancrofti,* the human parasite, than is the dog heartworm, but in the absence of comparative data on efficacy of drugs both species were tested. It was believed that more satisfactory conditions for testing would result if the age and degree of infection in the cotton rat were known. Hence, collateral to the studies in chemotherapy, an effort was made to discover an intermediate mosquito host for the cotton rat, but without success.

It soon became apparent that certain antimony and arsenic compounds were at least partially effective in treating the infestation in both the dog and the cotton rat, and studies of the metabolism of a large number of such compounds were undertaken. Since no satisfactory method for determina-

tion of small amounts of antimony in biologic material existed, methods that were of sufficient sensitivity for biologic application were developed in three separate laboratories.

In order to determine the comparative efficacy and toxicity in man of the commercially available antimony compounds previously used to treat various tropical diseases, including filariasis, a group of over one hundred persons infected with filariasis was treated intensively with maximal dosage of the compounds under study.[1] Four pentavalent and four trivalent antimonials were administered. Liver and kidney function and the blood count were followed, as was the level of antimony in the blood and urine of representative patients. Using as a criterion of cure the disappearance of microfilariae from the blood, the most effective and least toxic antimonials appeared to be the pentavalent compounds neostibosan, urea stibamine, and Stibanose (solustibosan). A similar but somewhat less extensive study of antimonials was also made in the Virgin Islands.

Since one investigator had found a new trivalent arsenic compound highly effective against heartworms in dogs and another had demonstrated its similar activity in the cotton rat, this compound was administered to patients with filariasis. It was effective in eradicating the microfilariae from the blood, but proved to be unduly toxic. It is expected that another new arsenic compound will be tried in man.

The survey of new nonmetallic agents in animals led to the discovery of a group of cyanine dyes capable of extraordinary activity against *L. carinii* in the cotton rat. Biochemical studies revealed that the activity of these dyes was due to their power to inhibit respiration of the filariae, which in turn resulted in inhibition of glucose oxidation and death. In the dog these dyes are not 100 per cent lethal to the heartworm but produce an effect on the worms similar to that of antimony; namely, a degenerative change in the uterus. Many of these dyes have proved quite toxic, but several of the most active ones are relatively safe and effective. These are now under study in two co-operating laboratories, which have explored approximately two hundred dyes in this series, and it is hoped that their efficacy against the human disease will soon be determined.

AMEBIASIS

The severity of the amebiasis infection acquired by the armed forces in Burma has been, if not surprising, at least disheartening. Use of all well-known therapeutic agents, whether singly or in conjunction, failed to produce permanent cures in any appreciable proportion of cases. This unfortunate situation stimulated the expansion of an experimental project al-

[1] These studies were made possible by the co-operation of the physicians at the School of Tropical Medicine in San Juan, Puerto Rico.

ready under way for screening new compounds in vitro and testing the more active ones in vivo in infected monkeys. Several encouraging leads have been uncovered. An arsenic compound less toxic and more effective than that now in use is being explored. One antibiotic, subtilin, gives promise, and one plant product is at least as active as emetine and less toxic. It is expected that at least one of these agents will come to clinical trial. Meanwhile the search for other agents continues under Army auspices. As a byproduct of this research, valuable information on the nutritive requirements of *Endamoeba histolytica* is being accumulated with a view to devising a synthetic medium for culture.

LEISHMANIASIS

Cutaneous and visceral leishmaniasis (kala-azar or Oriental sore), existing in Europe and Asia, were considered sources of possible widespread disease. No appreciable number of cases of kala-azar occurred, and it became apparent that this disease could be conquered by DDT and other preventive measures, but in the meantime considerable research on new chemotherapeutic agents had been undertaken. All available strains of Leishmania, including that from Khartoum, which is resistant to antimony, were imported and successfully inoculated into hamsters. Many hundreds of new agents were screened and the older antimony compounds were reassessed for curative power, but no effective new agent was discovered. Two pentavalent antimony compounds, solustibosan and urea stibamine, were found to be outstandingly effective. The latter had had a long clinical trial in India, but owing to the variability of the commercial product it was considered less reliable than certain other pentavalent antimonials. Efforts to synthesize a predictably homogeneous product were made in the United States but were not entirely satisfactory. Solustibosan had not been thoroughly explored clinically but was considered promising; its American form, Stibanose, was the least toxic of all the pentavalent antimonials and the most effective in the hamster.

The disease produced in the hamster by the Khartoum strain of *L. donovani* was closely followed. Amyloidosis, nephrosis, and death resulted in untreated hamsters. The toxicity, distribution, and excretion of the various antimonial drugs, with special consideration of the relation between tissue distribution and therapeutic effect, were determined. Development of a polarographic method modified to utilize small samples of biologic materials cleared the way for these valuable studies of the pharmacologic action of the trivalent and pentavalent antimonials in health and disease. Work continues on a small scale under sponsorship of the Army in search of an effective nonmetallic chemotherapeutic agent.

Phlebotomus diabolicus of Texas, which is the only species in that region

known to attack man, was collected and attempts were made to rear it in captivity. These were not sufficiently successful for it to be ascertained whether this species under favorable conditions might conceivably act as an intermediate host for kala-azar. Incidental to this project was the collection and description of a new species of Phlebotomus existing in the same region.

SCHISTOSOMIASIS

Despite an extensive survey of fresh-water snails in the United States, no species has yet been identified that is capable of permitting the complete development of *Schistosoma haematobium* or *S. japonicum*. A species collected in Louisiana by members of the United States Public Health Service was found to permit development of *S. mansoni* (the Puerto Rican strain) in a high proportion of exposed specimens. Other lots of these snails and specimens reared in the laboratory have been largely refractory to infection. This problem of biologic variation and the influence of environment make the scientist studying snails and other mollusks extremely hesitant to conclude that species refractory to infection in the laboratory would fail to become infected under ideal natural conditions.

The contractor responsible to the Committee on Medical Research for all the work on intermediate hosts of schistosomiasis has performed special service for all the investigators engaged in the chemotherapy of this disease. His laboratory has served as a reservoir for infected snails and animals and has shipped infected animals and snails to five other laboratories. This type of co-operation is typical of that exhibited by members of this group. Moreover, these studies would have been impossible without the aid of the Army and the United States Public Health Service. Members of the Army provided snails and dogs infected with *S. japonicum* on Leyte, and the United States Public Health Service generously shared all material and information received. Active assistance in importing and transporting infectious material for all these diseases was rendered by both services. When mice were in short supply, the United States Public Health Service prevented the halting of the entire program by arranging to supply the civilian laboratories.

Despite all efforts of civilian and government laboratories, it was found to be impossible to achieve a constant source of cercariae of *S. japonicum* for infection of animals on which drugs could be tested. The laboratories engaged in chemotherapy had to content themselves with testing the drugs against mice, hamsters, rats, rabbits, or monkeys infected with *S. mansoni*.

The purpose of the chemotherapeutic studies in this field was threefold: to discover whether different methods of administering the known therapeutic agents would be more effective; to investigate the effects of these antimony compounds on the host and parasite; and to canvass all types of new organic chemicals for better therapeutic agents.

It was soon found that the trivalent antimonials fuadin and tartar emetic were only partially effective in curing mice infected with *S. mansoni,* even when a toxic dosage was used. Monkeys and rats seemed to respond more satisfactorily to these compounds, but since the disease in the rat is self-limited, such results in that animal were open to question.

In the mouse (as has been reported for guinea pigs by Bang) the schistosome has been shown to leave the mesenteric veins during treatment with antimony compounds and to enter the liver. There was evidence that by intensive therapy the generative organs of the parasites were poisoned. During this period the feces were negative. Unfortunately this effect was not evidently permanent and regeneration sooner or later occurred. It may be that normal stools following such treatment in man are the result of a similar action of antimony on the flukes and that relapse may be expected later.

An evaluation was undertaken of pentavalent antimony compounds for treatment of schistosomiasis in man. Urea stibamine, neostibosan, and Stibanose were tested in Puerto Rico on patients infected with *S. mansoni,* and Stibanose was obtained for the Army for use on patients infected with *S. japonicum.* Urea stibamine alone showed promise. The excreta of 12 of 13 patients were negative for schistosome ova three months after treatment.

The biochemical requirements of the schistosome were measured and, surprisingly, these organisms were found to be similar to the filariae in their response to cyanine dyes. However, the fluke, unlike the filaria, has a reserve of stored carbohydrate and a system of anaerobic carbohydrate metabolism that renders it partially immune to the lethal effect; namely, the inhibiting action of the dye on the aerobic oxidative process. Further studies are in progress under Army support.

By good fortune certain members of several groups of aromatic nonmetallic organic compounds have, when fed to maintain a constant blood level, proved effective in 80 to 100 per cent of infected mice. These studies are also being extended and the appropriate series of compounds explored. Delay has been encountered as a result of the necessity for synthesis of related compounds. The active compounds are not appreciably toxic on preliminary test, and it is hoped that a new field for chemotherapy of this disease has been opened.

Further work in schistosomiasis should include the following: exploration of the biochemical needs of the flukes, with special reference to substances that may inhibit the anaerobic carbohydrate metabolism; further evaluation of the groups of new nonmetallic active compounds; and amplification of data on the metabolism of antimony and its effect on the body and the parasite.

METHODS OF DETERMINING ANTIMONY

The methods devised for determination of small amounts of antimony include colorimetry, polarigraphy, and spectrography. The colorimetric method, a modification of Frederick's rhodamine B method, is readily adapted to urine and, with further modification, to blood and tissue. It is sufficiently accurate to 2 gamma of antimony per gram. The polarigraphic method, adapted for use with very small samples of biologic material so that it is helpful in metabolism studies on small animals, is sufficiently accurate to 1 gamma of antimony per gram. The spectrographic method was devised only for urine and is capable of measuring as little as 0.1 gamma per milliliter. It is believed that modifications for analyses of blood would not be too difficult. This method of analysis was devised as a reference standard, but it cannot compete with the delicacy of measurements obtained through the use of radioactive antimony compounds. Both colorimetric and polarigraphic methods have proved to be extremely useful in the study of antimony metabolism.

SUMMARY

The achievements in this field may be summarized as follows. New chemotherapeutic agents have been uncovered for experimental amebiasis, filariasis, and schistosomiasis; the pharmacology and therapeutics of known antimony compounds have been elaborated; methods for determination of small amounts of antimony in biologic tissues have been devised; and light has been thrown on the biologic requirements of these parasites and the nature of the disease produced by each in the experimental animal. Future aims of the research program include clarification of the usefulness in man of the new chemotherapeutic agents that have been uncovered, elaboration of the biologic requirements of the various parasites, and further studies of antimony metabolism.

FUNGUS INFECTIONS

Fungus infections of the skin, even the ubiquitous "athlete's foot," are considered to be of meagre importance in civilian life, but in warfare, including modern mechanized warfare, fungus infections of the feet constitute a serious cause of disability. The tenacious behavior of tinea pedis as treated by conventional methods in the past is extremely familiar. The military services properly considered this disease worthy of serious attention. Fungus diseases in general are more prevalent in tropical areas than else-

where, and the prospect of combat in these regions enhanced their military importance. Two concurrent endeavors were made, one to test new fungicidal agents in vitro, the other to compare promising new agents with each other and with older accepted remedies in clinical studies, principally in military personnel.

A clue was found in the fact that bakers have employed in bread dough unsaturated fatty acids for inhibition of mold growth. Verification was soon obtained of the opinion that propionic acid and its salt effectively check the growth of various fungi and are quite nontoxic in the necessary dosages. Furthermore, they have a wide margin of safety and are indeed not harmful when administered parenterally.

A great number of in vitro tests showed that numerous strains of different types of pathogenic fungi can be inhibited or destroyed by unsaturated fatty acids. Fungicidal and fungistatic concentrations vary considerably, and the relative potency of different fatty acids is also variable, but the therapeutically effective concentrations were within a perfectly safe range.

Of the preparations studied, caprylic acid and its sodium salt seem somewhat superior to undecylenic acid and far superior to propionic acid preparations. Comparison with older remedies such as Whitfield's ointment shows that the fatty acids are less irritating and equally or more effective. Clinical trials have been extremely convincing, and commercial preparations, including liquids, pastes, lotions, and powders, are already available. Since most of these acids and their sodium salts can be given by mouth, their possible usefulness in the rare but serious systemic mycotic infections deserves investigation.

MEDICAL PROBLEMS OF CONVALESCENCE

EMMET B. BAY, JOHN E. HOWARD, AND E. COWLES ANDRUS

I MPROVEMENT in the surgical care of battle casualties and advances in the treatment of wound infections have notably decreased the mortality from injury or disease contracted in training or in combat. The modern methods of early treatment and the provision for attacking traumatic shock by means of blood and blood substitutes have undoubtedly saved many men who even a few years ago would have succumbed soon after injury. As a result of this decrease in early mortality, many men who would otherwise have died have entered on a prolonged stage of recuperation, and this in turn has brought into sharp relief the problem of accelerating or improving convalescence.

Advances in the treatment of wound infections, making possible earlier and more adequate reconstructive surgery, are described elsewhere. Military requirements have dictated that the health of the wounded or sick be restored as rapidly as possible so as to permit their return to active service. The remainder who cannot return to duty are entitled to the maximum benefits of medical care prior to discharge. These and other considerations called special attention to the particular problems of convalescence in the armed forces and to the rudimentary state of scientific knowledge in the general field. Some members of the armed forces were victims of infections, such as dysentery or malaria, and these presented a problem similar to that posed by the many patients in civilian life who are the victims of infectious diseases. In the armed services, however, there occurred with unprecedented frequency a situation in which men in the best of physical condition were suddenly struck down by injury.

It was obvious that adequate attention to convalescence and rehabilitation might be expected to redeem many patients from chronic illness and to relieve governmental institutions of the protracted care of a large number of disabled veterans. There was, in short, presented the challenge that the traditional methods of convalescent care either be confirmed by modern technics or be altered so as to expedite and promote the completeness of recovery.

The accepted medical practices regarding convalescence have in the past been largely empirical, and frequently, and in important respects, have

lacked the support of physiological evidence. A tendency had developed to apply specific therapeutic measures to the particular injury or identified illness and, with the exception of supportive measures, to leave recovery to the natural recuperative power of the body. The war stimulated a skeptical and detached examination of the accepted methods and turned attention to the period of sickness concerned with recuperation and to the nature of the factors common to convalescence from various injuries or diseases.

Under the first category, inquiry was directed toward evaluating the advantages or disadvantages of the long-accepted practice of subjecting the injured or the ill to prolonged rest in bed. Strangely enough, this seems never to have been carefully studied heretofore. It has been the conviction of the medical profession that optimum healing and the physiological readjustments that accompany recovery from illness depend on reducing the functions of particular organs. This has been translated into the practice of resting the patient as a whole. Moreover, although man is essentially a vertical animal, when he is injured or ill he is usually treated in the horizontal position. This has a firm foundation in the fact that man lies down to rest, and in many cases its benefits are obvious, but as can be demonstrated, prolonged confinement in the horizontal position can of itself work impairment of essential physiological adjustments.

Under two OSRD(CMR) contracts normal male volunteers of military age were put to bed for periods as long as seven weeks. To render the study more realistic, some of them were confined in plaster casts from waist to toes. The effects of this treatment on the circulation will be described in more detail below. Suffice it here to say that there was striking deterioration in the ability of these men to make the necessary circulatory adjustments when they were tilted to a nearly upright position. Moreover, they showed distinctive changes in their metabolism; there appeared a tendency to negative nitrogen balance, reversible by increasing the protein intake, negative potassium balance, negative calcium balance after the third week, and increased urinary excretion of riboflavin. The metabolic changes in themselves should not be taken as indicating that bed rest is disadvantageous. The circulatory consequences, on the other hand, are perhaps more significant.

METABOLISM

An important phase of the study of convalescence was the investigation of metabolism, particularly protein metabolism, following injury or illness. It has frequently been observed that during recovery or illness a patient almost invariably loses weight. With protracted illness wasting has always been a serious problem. It has sometimes been assumed that this is an inevitable consequence of disease, although its exact nature has been imperfectly understood. In some cases it could be accounted for in large measure

by the loss of protein-containing exudate from wound surfaces such as extensive burns. In others, disease of the gastrointestinal tract has imposed a certain degree of starvation.

The importance of this problem in relation to convalescence may perhaps be better appreciated by reviewing a few appropriate fundamental concepts. The unit of protoplasm of the body is the cell. Myriads of cells combine to form its organs and structures, and each cell has special functions to fulfill in the service of the whole organism. For its normal existence the cell must carry on with its immediate environment an exchange of essential substances, among which are the constituents of protein. In the normal process of metabolism body protein is continually in process of building and breakdown, and an equilibrium is established that represents the healthy function of protein metabolism. Obviously this cannot be observed in vivo in the cells themselves, but it can be followed in the organism as a whole by comparing nitrogen excretion with nitrogen intake. Under normal conditions this nitrogen balance may be temporarily increased or decreased by effort, fever, inadequate food intake, or overnutrition in proportion to body demands. It has been assumed that even under adverse circumstances protein wastage could be interrupted or diminished by increasing the protein intake. During recent years this has been facilitated by improvements in the available methods of administering the constituents of protein by injection.

The importance of maintaining a patient in positive nitrogen balance following operation or injury was to be assumed from observations already made by surgeons on the beneficial results of adequate preoperative and postoperative feeding. These conclusions were reaffirmed as the result of studies by other groups of investigators, who demonstrated by objective tests with ergograph and dynamometer the accelerated return of strength and endurance in subjects provided by mouth or by vein with adequate quantities of nitrogen in the form of amino acids. Others studied the fate of injected amino acids and devised methods for measuring their rate of excretion.

Sponsorship by the Committee on Medical Research of clinical investigation of the metabolic aspects of convalescence began with the support of a project originally directed to a study of the calcium metabolism in persons who had suffered fractures of long bones. Excessive loss of calcium in the urine had been observed in such cases, with the formation in some of renal calculi. In the process of conducting balance studies and metabolic survey of these patients, there emerged the significant observation that they also excreted extremely large amounts of urinary nitrogen. It was subsequently learned that similar observations had been made by Cuthbertson in Glasgow ten years previously.

Several other projects were directed to the study of metabolism in convalescence. Several widely separated groups turned their attention to the

subject, and members of these groups, with others not supported by the Committee, met frequently to exchange information and ideas. As a result of this collaborative study the following data regarding the nitrogen balance of patients with fractures have emerged.

In the first place, the evidence indicates that digestion and absorption of ingested protein proceed normally, since the stools do not contain more than the usual amount of nitrogen, approximately one tenth of the amount ingested. Following injury the patient gradually develops a deficiency of nitrogen, which reaches its peak at the end of the first week and then tapers off. This loss may reach the dimensions of 200 gm., which represents 7 kg. (more than 15½ pounds) of body substance as muscle. The nitrogen loss proceeds regardless of fever; indeed, it occurs even in patients who remain afebrile during the entire course. The loss of protein substance is not due alone to immobilization of the patient but is considerably increased by the fact of injury.

One group of investigators studied the problem of protein metabolism in patients suffering from various acute infections. They found that in meningococcal meningitis protein wastage might reach large proportions, and that in certain stages of the disease the protein losses could scarcely be reduced by the ingestion of maximal amounts of dietary protein and a high-calorie diet. This loss was observed to reach its maximum in eight to ten days after the onset of the illness and to vary somewhat from patient to patient. The amount of loss was not to be correlated with the temperature, nor was it influenced by intravenous administration of casein hydrolysate supplements.

Another investigator made an interesting series of observations concerning syphilitic patients with induced malaria. In the course of the ten bouts of fever usually administered for treatment, patients lost nitrogen in an amount equivalent to nearly 1300 gm. of protein. As these studies advanced this observer also noted the patient's ability to utilize administered protein constituents at various stages in this reaction. He discovered that the loss of protein per chill diminished with successive chills, being far less with the tenth than with the first. Furthermore, intravenous protein hydrolysate when administered at any time before the chill marking the abrupt rise in temperature was utilized in a normal fashion, but if it was given within a day after the peak temperature, a large proportion of the injected nitrogen was wasted. This phenomenon too diminished with successive chills.

Critical examination reveals that this reaction may follow a wide variety of stimuli. It is present to a certain degree in normal persons put at rest in bed. As noted above, it follows traumatic injury or infectious diseases, and it has also been observed after anoxia and hemorrhage. It has been described in normal persons undergoing acclimatization to a higher environmental temperature. Finally, it has been seen in patients suffering from severe burns. Here this reaction accounts for a wastage of protein in addition to

that lost by extravasation from the surface of the burn itself. Moreover, individual patients vary in their capacity to respond to such stimuli in this fashion. The ability to react appears to characterize the healthy person; the more debilitated the patient, the less intense is the reaction.

Progress has been made toward the development of methods of predicting the reaction of patients to surgical operation on the basis of a preoperative period of metabolic study. If during a period of four or five days' rest in bed on a diet containing 10 gm. of nitrogen and 1600 calories per square meter of surface area the patient remains in positive nitrogen balance, he is less liable to a pronounced protein catabolic reaction after operation than one who shows a negative nitrogen balance on such a trial.

On the surface it appears paradoxical that the proteins of the body of a robust man break down faster as a result of injury than those of an invalid, unless one assumes that the reaction represents a healthy capacity to mobilize resources for healing or repair. Its cause has not been found, but the suggestion has been made that it is the normal response to the absorption of some substances elaborated in the injured or diseased part.

ANEMIA

Primary anemias are not of great importance in military medicine, and the problem of acute blood loss was made less serious by the advances in blood storage and transportation facilities and by the massive blood-donor program so inspiringly supported by military and civilian personnel alike. However, the refractory secondary anemias associated with prolonged illness and chronic infections of various types still presented a special challenge to hematologists. The prospect of prolonged hospitalization of military casualties offered an opportunity to study such cases, and at the same time emphasized the need for knowledge on which might be based a rational approach to the therapy and reversal of the anemia. The newer knowledge of nitrogen metabolism during convalescence and the availability of such investigative tools and potential therapeutic agents as amino acids and new vitamins added to the promise of such studies.

Plasma-iron concentrations were studied at intervals after oral or intravenous administration of iron to various subjects. Normal persons and patients with iron-deficiency anemia responded with a prompt rise in serum iron and a gradual return to normal. In the patients manifesting the anemia of chronic infection, however, oral administration of iron caused no significant rise in the plasma-iron content (flat curve), whereas a single intravenous injection of iron caused a relatively small rise and a very rapid fall. It has been determined that there is no evidence of a defect in the synthesis of globin or protoporphyrin; indeed, there is an increase of the protoporphyrin in the erythrocytes and an increase in the coproporphyrin in the urine. At

the same time there is a significant decrease in the serum iron and an increase in the serum copper.

These findings suggested that there may be a defect in the metabolism of iron responsible for the well-known fact that cases of anemia secondary to infection do not respond to iron therapy until the infection has been abolished. Apparently in chronic infection a substantial amount of iron is diverted. There is some reason to believe that the available iron supply is plundered in order to maintain a high concentration in the inflamed tissues.

CARDIOVASCULAR ASPECTS

The studies of the circulatory system presently to be described were undertaken to aid the Army and Navy in the selection and discharge of personnel and in the handling of patients convalescent from a wide variety of illnesses and injuries. It was hoped that methods might be developed that would keep potential cardiac patients out of the services, make possible the rehabilitation of men discharged with a diagnosis of neurocirculatory asthenia, and shorten the period of nonactive duty of sick or injured servicemen.

To these ends, the Committee on Medical Research, with the advice of appropriate committees of the National Research Council (those on Convalescence and Rehabilitation, Cardiovascular Disease, Nutrition and Nutritional Disease, and occasionally others), made provision for the investigation of phases of the problems by competent civilians. Some of the contracts included aspects described elsewhere in this chapter. Some of the research required the co-operation of the Army, and in most instances this was excellent. The methods employed and the results obtained by the investigators may best be described under the headings indicated in the preceding paragraph.

How to keep potential cardiac patients out of the services. A group of prominent cardiologists undertook a statistical study of 22,741 medical records of Army officers in the Office of the Surgeon General. Their main objectives were to determine whether transient high blood pressure, transient rapid heart action, and obesity are related to the development of permanent high blood pressure and heart and kidney disease. They found them to be individually so related, and in combination to be roughly twice as significant in foretelling earlier disability or death. In other words, persons who have transient hypertension or transient tachycardia or who are overweight are more likely to develop sustained hypertensive vascular disease with all its implications than are persons with none of these abnormalities. If more than one of these predisposing factors is present, the chances of escaping hypertensive vascular disease are considerably less. Among the conclusions were the following:

(1) The decision as to the usefulness to the Army of a man with transient

hypertension (high blood pressure) will depend on the need for manpower. If this is urgent he may be accepted, provided his heart, arteries, and kidneys are normal.

(2) During times of peace, or whenever the need for men in the service is not acute, the transient hypertensive is not to be regarded as a first-rate risk; he may later prove a burden to the Veterans Administration.

(3) It seems probable that the facts pertaining to transient hypertension that have been derived from an analysis of the records of Army officers apply also to the general male population of comparable physical fitness and similar age groups.

Since high blood pressure is such a common disease, it is probable that this study will have greater significance for civilian medicine in the long run than for guidance in selection of military personnel, although it is obviously important to the Army, Navy, and Veterans Administration.

How to rehabilitate men discharged with a diagnosis of neurocirculatory asthenia. Thousands of men were discharged from the services during and after World War I with a diagnosis of neurocirculatory asthenia. Many of them are still being cared for by the Government as totally and permanently disabled. Anticipating a similar situation in this war, and because there had been such disagreement between various examiners in the rejection of 686 men (1.14 per cent) of 60,000 draftees for this cause, another group undertook a fresh study of the disorder. The psychiatric aspects of their investigation are presented elsewhere in greater detail. The physiological studies were for the most part directed toward detecting abnormalities of the circulatory system. These were few in number. The subjects responded to various exercise tests as though they were in a poor state of physical training, although most of them were or recently had been in the Army. Thus, they averaged significantly higher blood-lactate levels on moderate exertion than did normal controls. Their blood-lactate levels did not, however, reach those of the controls when they were asked to perform heavy work, because they would not work nearly as hard as the controls. They were hypersensitive to painful stimuli, and refused to attempt physical training because of the uncomfortable sensations attendant thereon or the fear that this might make them worse.

It was finally concluded that neurocirculatory asthenia is chiefly a psychiatric disorder divisible into acute and chronic forms. There is some evidence that the acute form responds to treatment if not merely to the passage of time. The therapy of the chronic form is admittedly difficult, but at least the proper approach is now known. Fortunately the incidence of neurocirculatory asthenia in the armed forces has been much lower than was expected. The elucidation of the disease will undoubtedly benefit civilian medicine.

How to shorten the period of nonactive duty of sick or injured servicemen. Little was known concerning the convalescent period of most disease states.

Indeed, the rate and degree of physical deterioration during, and of recovery after, prolonged rest in bed were unknown. There were indications that proper food, suitable exercises, and other aids might shorten the convalescent period in many cases. The problems assigned to several groups of investigators in this field concerned the measurement of physical, including chiefly circulatory, changes occurring during convalescence and their modification by attention to the patient's metabolism and activity. The metabolic aspects have been described above. It should be noted that several of the groups carried on both types of study.

As a necessary control for the studies on illness and injury, two groups of investigators were awarded contracts for research on the physiological changes accompanying simple rest in bed. Both groups used conscientious objectors as subjects.

One group found that there was a reduction in heart size (11 per cent), in blood volume (10 per cent), and in oxygen transport per heart beat during work, but no change in thermodynamic efficiency while walking or running. Such reduction in co-ordination as might result did not alter the oxygen cost per unit of external work until further inco-ordination was produced by fatigue. An especially interesting, if unexplained, finding was that interruptions in the schedule of reconditioning exercise (after bed rest) produced a marked temporary relapse in physical condition, which did not occur with interruptions in a simple conditioning exercise schedule not preceded by bed rest. Manual speed and dexterity were unaffected by bed rest, with or without surgery, but postural stability was affected by it to almost precisely the same extent whether or not surgery was performed. Instability increased by about 50 per cent on the first day out of bed.

A second group of investigators modified the above experiments by applying casts from the waist to the toes of healthy men placed on bed rest for six weeks. The circulatory changes noted were in general agreement with those observed by others studying this problem. One of the outstanding observations was that bed rest brought about a marked decrease in the tolerance of the body to tilting 65° with the feet downward for twenty minutes, subjects tending to faint on tilting during bed rest. This was accompanied by a change in pulse rate and pulse pressure and by the development of purpuric spots on the skin of the feet and legs. The circulation time and red-cell count did not change during the period of bed rest, but the blood volume decreased. Physical efficiency as measured by the Master two-step test and the Schneider test fell off approximately 20 to 25 per cent after the period of rest.

Using patients recovering from infectious diseases or surgical operation, a group of investigators studied several circulatory and respiratory functions in the basal state. These studies included estimations of arterial and venous blood pressure, blood velocity, cardiac output, transverse diameter of the

heart, basal metabolic rate, ventilation, coefficient of utilization of oxygen, blood volume, and plasma proteins and a record of the electrocardiogram. None of these observations indicated any characteristic abnormality when compared with currently accepted normal standards.[2] Follow-up studies revealed small but distinct alterations in some of these functions when the patient was compared with himself as a control. The most important changes during convalescence were a deficit in blood volume of approximately ½ liter and a venous-pressure deficit of 35 per cent.

The studies of blood volume were of particular interest because these investigators were able to use both the plasma dye dilution method and the radioactive iron-tagged red cell method. Incidentally, a new device, a timer for x-ray films of the heart in systole and diastole during tilt-table experiments, was developed.

Others studied the metabolic and circulatory responses of patients to surgical operations. They found an increase in the standing pulse rate on the tenth postoperative day and an increase in cardiac output in the vertical position. Exercise of these patients while in bed resulted in less tremor on standing for the first time after operation and a slighter rise in pulse rate, but the differences between the exercised and the control group disappeared within two or three days after the patients became ambulatory. There was an increase in oxygen consumption during postoperative exercise beyond that produced by the same exercise before operation. In surgical patients in whom an effort was made to maintain nitrogen balance, various circulatory tests, including tilt-table experiments, showed that the deterioration of these circulatory functions tended to be less in patients who could be kept in nitrogen equilibrium.

In another project the nature and degree of physical deterioration provoked by artificially induced malaria were carefully analyzed. It was observed that lactic acid produced during exercise, probably one of the limiting factors in effort, accumulated more rapidly and, for the same effort, in larger quantities in malarial subjects than in others.

Other investigators devised an exercise test in which patients lifted a 40-pound weight sixty times in two minutes. Usually after the third postoperative day the pulse rate after exercise had returned to the preoperative level. They also used a step-up test and the tilt table, obtaining results comparable to those of others, except for the fact that the response of the pulse pressure and pulse rate to tilting returned to normal on the fourth postoperative day.

Circulatory, metabolic, and psychological aspects of convalescence were studied in patients at the Army's Gardiner General Hospital in Chicago by a team of collaborating specialists. In soldiers convalescent from a wide

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[2] The suggestion is made that some of the latter may have been developed from data obtained from convalescents, and that they are adequate for most clinical purposes because pathologic changes are sufficiently large.

variety of illnesses and injuries they measured oxygen consumption and heart rate during mild exercise in bed, during which the steady state was achieved. They derived the formula $\dfrac{\sqrt[3]{\text{oxygen consumption}}}{\text{heart rate}} \times 1000$, which remained quite constant at different levels of work between consumptions of 400 to 1000 cc. of oxygen per minute per 1.75 m² of body surface. This ratio is relatively large in normal subjects and small in patients who are ill, except in cases of infectious hepatitis. The ratio tends to increase as the patient improves clinically.

In a single patient studied over a thirty-week period it was shown that there was a variable response to an exercise-tolerance test on certain days of the week. Stress-producing situations of daily life occurring during the course of a highly routinized work week were reflected in predictable changes in cardiovascular function.

A new device for measuring circulatory and perhaps physical fitness was tested on normal men and convalescents. This was based on a spectroscopic estimation of the time required for the disappearance of the oxyhemoglobin band from the spectrum of the skin of the hand after occlusive pressure had been applied to the arm, while the subject breathed normally and again while he held his breath. The physiological factors implicated in this simple test are complex and not yet completely understood. Empirically and on a statistical basis, however, it is clear that the test has validity in grading physical fitness. The conditions under which it is made have to be carefully controlled, but when they are, retests have a correlation coefficient of about 0.80. Blood donors showed a significant drop during the first twenty-four hours and patients with malaria a low value during the first two weeks of recovery. The test needs further validation in the convalescent state, but it may prove to be a valuable tool.

In summary, scientific attention has been newly focused on the convalescent state. The practical results of this attention for the war effort were meagre, partly because the problem proved to be extremely complex and partly because the war ended before some of the discoveries could be applied. Most investigators agreed that tests of circulatory fitness offered the best hope for measuring progress during convalescence. None was sure that any one test could be adopted as the sole guide for the physician managing the convalescent patient. Many of these studies are continuing under the auspices of private institutions. They may be confidently expected to influence favorably the duration and completeness of convalescence from disease or injury.

PSYCHIATRY, INCLUDING THE
PSYCHIATRIC ASPECTS OF CONVALESCENCE

The psychiatric problems engendered during the recent emergency have been widely described and widely appreciated. In fact, they constituted an emergency in themselves. The responsible medical officers of the armed services were faced with an overwhelming need to apply preventive and therapeutic measures to the large number of men who broke down under the strain of military training or combat. A genuine attempt was made by the Selective Service to screen out men likely for psychiatric reasons to become ineffective. No doubt this did prevent the acceptance of many who would otherwise have been added to the load of psychiatric casualties, but considering that those responsible for the psychiatric examination at the induction centers could spend at most a few hurried minutes with each man, whereas in civil life a psychiatrist requires an hour or more to evaluate the psychiatric status of a patient, it is not wondered at that many potential casualties were overlooked.

Few of the civilians who entered the Army or Navy had encountered the type or degree of strain that they met in the armed services. These were many and varied. They rested on the necessity of uprooting free young people from their civilian and home environment and subjecting them, without reference to their own choice, to surroundings, disciplines, and dangers to which they were unaccustomed and for which they were unprepared.

The problem was promptly recognized by the Army and Navy. Outstanding psychiatrists were recruited from civil life who applied themselves energetically to the operation of a service that would provide the means of recognizing impending psychiatric breakdown and of instituting early or preventive treatment. Psychiatrists were attached as consultants to the staffs of the service commands, and as many as possible were provided in the training centers. Particular effort was devoted to making the medical officers who were not specialists familiar with the early manifestations of psychiatric abnormalities and with the most effective means of treatment. The armies in the field and the naval units abroad were supplied with psychiatric advice, and as far as possible with officers specially trained in this particular field, but the supply of well-trained psychiatrists was far short of the need. A most important limiting factor in the provision of psychiatric diagnosis and treatment is the time required for its adequate practice. Personality patterns are infinitely variable; the accepted normal merges at its border with the abnormal to such a degree that, in contrast with at least some of the results of medical examination, psychiatric data defy quantitative measurement. In spite of the limitations imposed by the lack of trained personnel and by the press of routine duties, however, outstanding psychiatric studies were made by officers of the Army or Navy and have been reported.

The personnel requirements of the services brought into sharp relief the fact that there were far too few well-trained psychiatrists in this country. As has been mentioned, the armed services acquired fewer specialists in this field than their needs dictated. Those whom they did acquire included some of the outstanding members of the profession and left those remaining in civilian life busier than ever, overburdened with the demands of clinical practice and teaching and, with a few fortunate exceptions, not available to conduct or direct projects of research. Opportunities for clinical investigation by civilian psychiatrists of cases of psychiatric breakdown induced by the rigors of training or combat were necessarily limited. Practically all the psychiatric casualties occurring in the armed services were hospitalized in special Army or Navy establishments. In several outstanding instances, however, psychiatrists in practice or on the faculties of medical schools acted as advisers or consultants to neighboring installations for the care of such patients, and they were occasionally able to collaborate in research stimulated by the problems encountered in these institutions.

The lack of adequate numbers of trained psychiatrists had a significant consequence in stimulating the evaluation and employment of group psychotherapy. By this means, after a certain amount of preliminary individual discussion, patients can be handled in groups. In addition to saving time, the method has the special advantage for some patients of demonstrating that their problems are not unique to them.

The number and variety of possibly useful studies that could be made by civilian psychiatrists in civilian institutions was sharply limited by the number of competent investigators. This is reflected in the fact that only eight projects in this field were sponsored by the Committee on Medical Research. Two were directed to the development and testing of methods for assaying the neurotic potentialities of individuals and the likelihood that they would succumb to the strains imposed by military service. One of these projects was devoted to evaluating under military conditions certain proposed improvements in the methods of association-motor studies. Normal enlisted men were studied in comparison with psychiatric casualties, successful pilots, others who had failed during pilot training, and recently inducted aviation cadets. The results failed to demonstrate the value of the suggested improvements and confirmed the accuracy of the original (Jung) association studies. Other investigators applied to large numbers of normal men, psychiatric casualties, and persons convalescent from minor illnesses various psychological tests already developed and others newly designed. These trials led to the development and application of a standard method for the assay of existent or impending neuropsychiatric and psychosomatic disturbances. This method has been published as the "Cornell Index" and has been widely used in induction centers and Army and Navy installations for the prediction of psychiatric disorders and the evaluation of methods of treatment and

prevention. It has already been administered to thousands of persons and has contributed to the quick and efficient sorting of the functional and organic components of disease in casualties.

Another project was supported to follow through service in the Army a significant number of men whose psychiatric histories were matters of record in certain university health centers. This study has elicited the information that a relatively small percentage of those accepted for military service who had previous records of what was at the time taken to be serious psychiatric abnormality were discharged for psychiatric reasons. A few of these men, indeed, achieved brilliant military records. This study is continuing under Army auspices, and it is probable that it will supply information of fundamental importance for the evaluation of the validity of some of the present concepts about officer selection and the role of the psychiatrist in problems connected with the utilization of manpower.

Under a fourth project there was conducted an exhaustive comparison of the results of medical, psychiatric, and social analysis of 100 neuropsychiatric patients in Army hospitals as compared with 100 supposedly normal soldiers. This disclosed that the vast majority of the psychiatric casualties had evidence of illness five years or more prior to entry into the service. The majority of these patients also entered the hospital with various somatic (physical) complaints, indicating the importance of considering the psychiatric elements of illness.

Two other projects covered the identification and evaluation of psychiatric factors that would contribute to the prolongation of convalescence from injury or disease. One of these was suggested by a separate study that dealt with the results of head injury, which had shown that the duration and completeness of convalescence after such injury were notably influenced by psychiatric factors. Outstanding among these factors were the apparent adjustment of the patient to environmental conditions prior to injury and the development of tension or anxiety neuroses following it. Investigation was directed to patients who did not recover satisfactorily after disease, injury, or operation. The patient's ability for sustained mental effort proved to be significant in predicting the progress of his convalescence. This study also investigated the results of intensive individual psychotherapy among patients showing delaying convalescence.

Another project concerned the study of changes in cardiovascular function associated with emotion, acute disease, and the convalescent state. Objective measurements were made of various circulatory phenomena in normal subjects and in selected patients at rest and after exercise. These were repeated in association with imposed situations that provoked emotional response. Special attention was paid to symptoms of fatigue experienced on moderate exertion and apparently having to do with the attitude and motivation of the subject toward the task to be performed. The results indicate that the efficacy

of the circulatory functions of normal persons may be profoundly and adversely influenced by emotion.

If there are psychiatric hazards involved in the induction of large numbers of young men into the armed services and their training and combat experience, there are also essential psychiatric problems demanding study and attention among persons rejected for military duty or discharged for psychiatric or medical reasons. One project that was closely integrated with other studies conducted under the Mental Hygiene Association concerned the psychiatric rehabilitation of discharged servicemen. A particular contribution of this project was an evaluation of group psychotherapy. Several hundred cases were registered and investigated. It was demonstrated that about half the cases could be handled effectively by brief periods of psychotherapy and that group psychotherapy was feasible and valuable. Incidentally, the experience of the investigators in this project underscores the necessity of the closest co-operation between social agencies in the community, the Veterans Administration, and the Office of Vocational Rehabilitation. This work is continuing under the auspices of the Mental Hygiene Association.

Finally, and perhaps belatedly, a study was begun to identify and evaluate the psychological factors that oppose the full rehabilitation of injured or mutilated persons and to make a beginning toward this treatment. Under this project investigators studied the sociopsychological factors influencing family, friends, or strangers in their reaction to amputees or to patients requiring extensive cosmetic plastic surgery, and those affecting the attitude of the injured themselves toward other injured persons and toward their family, friends, or strangers. In this direction only a beginning has been made. Work under this project is continuing with support from the Army.

SPECIAL COMMISSION TO STUDY COMBAT EXHAUSTION

Repeated informal conferences with the Psychiatric Consultants Division of the Surgeon General's Office of the Army demonstrated the desirability of a careful and deliberate study of psychiatric casualties developing under combat conditions at the earliest possible stage, preferably as far forward as the casualty clearing station itself. It was agreed that there might be essential differences between the psychiatric conditions manifested in acute combat casualties and those experienced in the typical psychoneurotic reaction of civilian experience, and that the condition might change so rapidly that the impression gained by a study as the soldier was evacuated toward the rear would fail to reveal significant characteristics present in the earliest stages of the abnormality.

Obviously, accurate knowledge of the pattern of development of the psychiatric picture is essential in order to plan and guide the most effective treatment. Since it was emphasized that the psychiatrists in the Army, par-

ticularly those at the combat level, did not have time to undertake such a deliberate study as would be necessary to acquire this essential information, the facilities of the Office of Field Service of the Office of Scientific Research and Development were described to the Psychiatric Consultants Division in the Office of the Surgeon General, and as a result, on April 20, 1945, a commission of five civilian psychiatrists departed for the European Theater of Operations.

The principal objectives of this commission were to study the psychodynamics of combat exhaustion and to evaluate or devise procedures and policies regarding such disabilities in the light of the insight so obtained. At that time, however, the conditions most productive of combat exhaustion no longer generally prevailed. The front was highly mobile, and a sense of victory was in the air. Although the drive into Germany was extremely fatiguing, it was emotionally a far different experience from that involved in the costly attacks on the heavily fortified positions of the western defense lines, where men were often mown down by rifle and machine-gun fire or blasted by mortar and shell fire in a terrain carefully and deceptively prepared for defense in depth. At the time when the commission reached the First Army, however, there were again numerous psychiatric casualties from the Leipzig area, which was heavily defended by "ack-ack" artillery against our advancing infantry. The members of the commission had opportunities to study such fresh cases in the First Army's treatment center and later in Weimar, and also to investigate some of the changes that occurred during prompt recovery and during the progressive development of neurosis in cases in which recovery was not so prompt. Cases of special interest here were junior officers who had for months successfully led fighting units in action through numerous cycles of heavy losses, replacements, new heavy losses, and fresh replacements, and who showed the emotional strain involved in repeatedly commanding into fatal action men closely bound to them in the peculiar intimacy of the small combat unit.

The members of the commission, since they were considered "not expendable," had no opportunity for such immediate personal experience of violent combat activity as would have been appropriate for the fullest appreciation of the situation in which combat exhaustion occurred most characteristically. They did, however, have the good fortune to meet with the fullest co-operation in all installations visited, and thus had invaluable opportunities to benefit from the experience of many psychiatrists and other medical experts who had dealt with these psychiatric problems at all stages and in many different settings.

The opinions thus gathered varied widely and appeared to be chiefly determined by whatever segment of the problem the individual physician had seen. There is a sort of fractional distillation of cases of combat exhaustion from the front to the rear; the men who are most responsive to treatment

make quick recoveries and return promptly to duty, whereas those less responsive continue toward the hospitals in the rear. In the prolonged cases there is a strong tendency toward a progressive development of guilt-laden depressed states or of the chronic neurotic reactions well known in civilian psychiatry as unconscious defenses against a sense of guilt. Nearer the front, there was a large proportion of acute, clinically amorphous conditions with quick recovery, representing the reactions of men of normal personality structure to highly abnormal situations. Psychiatrists whose work had been in base hospitals naturally had experiences and therefore opinions different from those of specialists who had worked with the more responsive cases near the front.

In July 1945, the commission prepared and filed a report of its study and made recommendations intended to be helpful in the Pacific Theater, but the war concluded there also within a few weeks. The report may therefore now have its principal value in enlightening psychiatrists who deal with the later sequelae of combat exhaustion. Some of the commission's observations and conclusions are summarized below.

All the widely varied acute psychiatric conditions occurring in combat were for practical purposes labeled "combat exhaustion." Fatigue appeared to be an important contributory cause of reduced capacity to withstand emotionally disruptive influences, but this was not in itself an adequate cause of the typical psychiatric disability of combat. Severe or moderate anxiety states, existing prior to military service, seemed to be one of the personal factors predisposing to combat exhaustion. Emotional immaturity was also a factor in vulnerability, particularly in the first combat experience. Pre-existing mild neurosis did not appear to be of crucial importance in the primary occurrence of combat exhaustion, but was influential in shaping the neurotic pattern in its clinical evolution when prompt recovery did not occur. Familiar forms of neurotic reaction, such as hysterical disability, did occur at the onset in some cases, but there appeared to be an extremely large proportion of cases quite unlike the neuroses seen in civilian life or in Army training camps.

The commission concluded that the intensity of combat appeared to be the main factor in the production of combat exhaustion. This factor seemed to operate in a number of converging ways. Emotional conflicts were intensified and anxiety was increased. Furthermore, high casualty rates removed trusted leaders and comrades, and the green replacements were often unsure of themselves, distrusted by the veterans, and lacking in esprit de corps. The dispersion of the small combat team in foxholes, necessitated by intense enemy fire, lessened the group feeling; thus there arose not infrequently the erroneous impression, "My whole outfit has been wiped out." All these factors tended to raise psychiatric casualty rates disproportionately in periods of intense and prolonged combat.

In their studies of individual cases, the members of the commission were particularly impressed by the degree to which the group integration of the small combat unit had enabled men to endure enormous emotional stresses. Conversely, they were impressed by the disastrous results in the form of individual collapse and incapacitation that occurred when a small unit was emotionally or physically disrupted. The importance of fear, the commission believed, has received too exclusive attention in the consideration of the psychodynamics of combat exhaustion. Aggressive tendencies, strongly aroused in combat, constituted a disruptive emotional force difficult to keep focused exclusively on the enemy. The prevalence of irritable reactions, so marked a feature in many acute conditions, was only one — but a significant one — of the evidences of the psychodynamic implications of resentment and hostility. The maintenance in combat of a steady pattern of close in-group confidence and support is a crucial factor in preventing psychiatric disability.

A number of types of treatment were observed — barbiturate narcosis, hypnosis, chemically induced abreaction, sub-coma insulin, group therapy, and so forth. Successful treatment seemed to depend less on specific procedures or drugs than on general principles. Chief among these was that of promptness in providing rest and firm emotional support in a setting in which the bonds of comradeship with one's outfit were not wholly disrupted and in which competent psychiatric reassurance was fortified, symbolically and physiologically, by hot food and clean clothes and by evidences of firm military support and command of the situation.

The members of the commission were particularly interested to learn about the subsequent effectiveness of soldiers who returned to combat duty after suffering combat exhaustion. Spectacular recoveries sometimes occurred. Men who were returned to such duty by competent and experienced psychiatrists had subsequent records of good service not markedly different from those of controls, but the results are somewhat confused by the fact that psychiatric recommendations against reassignment to combat duty were not infrequently disregarded by personnel officers, with recurrence of the disability and consequent danger and trouble to the sick man's comrades in getting him back to a treatment center. The commission heard impressive accounts of men recovered from combat exhaustion who had absented themselves without leave from replacement depots in order to be sure to get back to the front with their own combat units.

The commission emphasized in its recommendations the importance of preventive measures depending on command functions, such as policies of rest and rotation and the cultivation of better group spirit and leadership, particularly in reinforcements.

Part Two: Surgery

INTRODUCTION

JOHN S. LOCKWOOD

ADVANCES in surgical management of wounds have always met their greatest test in time of war. In the course of a single engagement, the military surgeon is likely to be called on to treat as many severe wounds of violence as he could expect to encounter in several years of civilian practice under peacetime conditions. To be of service to the surgeon in the area of combat, new technical procedures must require no material that is not readily available even under the difficulties of military supply; they must be relatively simple in their application, so as to be adaptable to the level of competence of the average rather than the exceptional surgeon; and they must not subject the patient to the slightest added risk of losing his life simply in order to offer him a comparatively superficial gain.

In the light of these requirements, it was necessary that the objectives of wartime research on problems arising from combat injuries should be concerned not so much with the evolution of new basic concepts as with the development and testing of procedures designed to make available to the military surgeon the detailed practical application of fundamental knowledge already in existence. The work of civilian investigators was bound to suffer certain limitations imposed by the necessity of working under conditions that never duplicated those of combat: a method of treatment that may appear to be valuable when tested in the experimental animal may be without merit when applied to a human wound; the accidental wound sustained under conditions of civilian surgical practice is bound to differ in many important respects from the wound sustained in battle; what is a practical procedure under civilian conditions may be entirely impractical under the special conditions of military surgery. The success of the civilian research program on wounds must therefore be measured in relation to these stated objectives, and, in those respects where it has failed, allowance must be made for the difficulties that have been enumerated. Some of the accomplishments to be described in this section never reached the level of practical use-

fulness for the Army and Navy surgeon, but will be nonetheless valuable in peacetime civilian practice.

The first consideration in the treatment of the wounded soldier is the saving of life. Perhaps the outstanding accomplishments in military surgery during this war were the improvements in surgical resuscitation made possible by the availability of blood and plasma for transfusion in the forward areas, coupled with early surgical intervention. Both these measures contributed greatly to the prevention of early deaths from blood loss and shock. However, their effective performance was not so much related to new knowledge gained through wartime research as to the successful solution of the problems of logistics involved in applying knowledge that had already become a part of the best civilian surgical practice.

Second in importance to the prevention and treatment of shock, but closely related to it, is the control of infection in wounds of battle. It is difficult to calculate the precise toll of death and disability during World War I directly attributable to infection, but all observers agree that the sum total was enormous. The introduction of the sulfonamides during the five years immediately preceding the outbreak of the last war and of penicillin in 1942 presented a challenging opportunity for research designed to determine the best methods of applying these new agents to the problem of infection in war wounds. This section tells the story of the large-scale efforts that were made by civilian investigators, working with funds and facilities provided by the Committee on Medical Research, to determine the possibilities and limitations of the new chemotherapeutic agents in relation to this phase of surgery. The execution of this program required, and received, a measure of teamwork that was almost unprecedented.

Although the usefulness of the sulfonamides, and especially of penicillin, in controlling fatal invasive infections was clearly established, it early became apparent that the chemotherapeutic agents were of limited usefulness in preventing local infections of wounds, and that in this particular sphere early and proper surgical treatment of the wound remained the weapon of greatest usefulness. At the same time, it was found that the proper combination of surgery and chemotherapy would permit the surgeon far greater latitude in carrying out reconstructive procedures in patients with infections of soft parts, bone, and body cavities, and that the time required for final and permanent healing of such wounds could be greatly reduced.

The problem of burns received special attention from civilian investigators. At about the time the war started, the weight of evidence had begun to accumulate against the tannic acid method of treatment, which had been widely employed by civilian surgeons for fifteen years. It soon became clear that the Army and Navy surgeons urgently required a new method of burn treatment that would be better adapted to military conditions than the means then available. Since shock represents an important aspect of burns, as well

as of penetrating wounds, it was necessary that the new method of burn treatment should embrace the general physiological disturbance of the patient, as well as the problems related to the burned tissues themselves.

One of the outstanding accomplishments in surgery during the war was the development and large-scale testing of the pressure-dressing method, which, coupled with improved control of shock, resulted in the saving of lives of men with burns of such extent and depth as had been uniformly fatal during the preceding era. The factor of infection in burns came largely under control and, together with new methods of hastening the process of healing, afforded a substantial reduction in the amount of disability and disfigurement. The steps leading toward placing burn management on a sound physiological basis are described in Chapter XII.

The wounds of warfare frequently result in serious damage to nerves and brain tissues of such highly specialized significance in the body economy that their successful repair becomes one of the major responsibilities of military surgery. It was necessary not only to obtain greater insight into the nature of the reparative processes of nerve and brain tissue, but also to discover new methods of restoring nerve function to extremities that had suffered such major losses of nerve tissue as to make final restoration of continuity all but impossible. In the same general sphere of study was the problem of concussion of the brain, particularly the physical factors involved in its production, its pathologic nature and prognosis, and methods that might be developed to prevent it or to moderate its permanent consequences. This program of research required the co-ordinated efforts of neurosurgeons, neurologists, anatomists, and physiologists, and ultimately achieved a close measure of collaboration with work going on in the military and naval neurosurgical centers.

A number of special problems came up for study: the nature, prevention, and treatment of frostbite and trench foot resulting from overexposure to cold, and the development of new and better surgical sutures, improved materials for controlling hemorrhage, and devices for x-ray photography that would eliminate guesswork in the production of high-quality films. Each of these problems was taken up by one or more teams of investigators, with results of immediate practical significance that are described in the various chapters of this section.

The research on wound ballistics had a twofold objective: first, to obtain precise quantitative definition of the mechanical factors involved in production of missile wounds; and second, to provide the surgeon with a clearer understanding of the extent and nature of the tissue injury produced by various types of missiles — information likely to be of great value in planning and executing adequate surgical treatment. The results of the combined efforts of physicists, anatomists, biologists, and surgeons in clarifying some of these problems furnish a striking testimony of what the technics

of modern scientific research can achieve toward clarification of little under-stood phenomena, in which man's interest is as ancient as the art of war itself.

Running through this entire section is the evidence that surgical research cannot be isolated from research in related fields of general biology and medicine. The Committee on Medical Research established a Division of Surgery for purposes of administrative convenience. When, however, this division was confronted with need for work on the fundamental aspects of wounds and wound healing and the physiological aspects of various types of injuries and their reparative processes, it became imperative that a major portion of the substantive work be borne by persons with no permanent orientation in the field of surgery; the major role of the surgeons involved in this program was to keep the efforts of all related personnel focused on problems of importance to the military surgeons in the field. If the many surgeons who participated in this program were successful in doing more than that, it was not simply because they were skilled in surgical technics, but also because they were to some extent qualified as investigators in one or more of the basic fields of medical science. Thus, the emphasis that modern surgical teaching has placed on the general scientific qualifications of the student and teacher of surgery appeared during this war to achieve full justification, not alone in the military hospital but in the research laboratory as well.

THE PREVENTION OF INFECTION IN ACCIDENTAL WOUNDS

FRANK L. MELENEY

OBJECTIVE

SOON AFTER war was declared in Europe in 1939, it became obvious that the United States would sooner or later become involved. In May of 1940, with the collapse of France and the overrunning of the low countries and Norway, England alone stood between the German juggernaut and our own shores.

The Surgeons General of the Army and Navy called on the National Research Council for advice regarding all the latest developments in medical science that might be of aid to them in the prevention and treatment of disease and infection. Among other things they asked for advice regarding the use of the newer chemotherapeutic agents, particularly the sulfonamides. Various committees and subcommittees were appointed to respond to this call. The Subcommittee on Surgical Infections applied itself to the problem of the prevention of infection in war wounds and burns. Such activity was essential, for this was not a simple problem that could be settled on theoretical grounds alone.

The most successful response to the sulfonamide drugs was in the field of medical infections, which are characterized by a diffuse cellulitis without any breakdown of tissue. Drugs administered systemically can reach such tissues by the blood stream, and when resolution takes place the part is restored to normal. It was early observed that these drugs did not do so well in surgical infections that are characterized by a breakdown of tissue or a localized confined exudation of pus. Nevertheless, it was hoped that they would be of great aid in the prevention of infection and thus cut down the number of cases, and would shorten the duration of disability, hasten healing, enable prompt return of injured men to duty, and minimize the fatalities resulting from war wounds.

METHODS OF STUDY

Recognizing the fact that no accurate knowledge was available with respect to war wounds, the Subcommittee on Surgical Infections decided to

take stock of its present knowledge. The following facts seemed to be well established.

All wounds, whether accidental or military, are contaminated by bacteria. There is nearly always some damage to deep tissues, with extravasation of blood. Sometimes there is tearing of muscle and fracture of bone. The growth of organisms is favored by the presence of injured tissue and foreign bodies. Certain organisms enter the wound when it is infected; others contaminate it later for as long as it remains open. Any organism that enters a wound may find conditions favorable to its growth, enabling it to colonize and produce its poisons; this may take place as early as two to three hours after contamination. The motion of any part containing infected tissue increases the activity of the infection. Surgical débridement will remove a large proportion of the contaminating organisms, foreign bodies, and damaged tissue. Such wounds cannot be completely sterilized by any known procedure, but the proportion of organisms removed by débridement depends on the care employed and the period of time after contamination that débridement is performed.

Sulfanilamide may be taken by mouth in doses of 6 gm. initially and 6 gm. every twenty-four hours at four-hour intervals without serious consequences in most cases, and this dosage will maintain a level in the blood of approximately 5 to 10 mg. per 100 cc. as long as it is given. Sulfapyridine may be so administered, but it is extremely nauseating, and a constant blood level is much more difficult to maintain because of its variable and generally slower rate of absorption. Sulfathiazole may also be administered in this fashion and is less nauseating than sulfapyridine, but the maintenance of a constant blood level is even more difficult. Sulfanilamide when given by mouth is particularly effective against the hemolytic streptococcus and will prevent its spread from almost any portal of entry. It is relatively less effective against other organisms. Sulfapyridine when given by mouth is effective against the hemolytic streptococcus and pneumococcus, but it has relatively less effect on other organisms. Sulfathiazole when given by mouth is effective against the pneumococcus and to some extent against *Staphylococcus aureus,* the hemolytic streptococcus, and *Escherichia coli,* but also has relatively less effect on other organisms.

The effect of these drugs, when given by mouth, on the gas-gangrene and tetanus clostridia and the anaerobic gram-negative bacilli and streptococci is variable and uncertain. Their bactericidal and bacteriostatic effects are handicapped or inhibited by the presence of pus or exudate or of split products of protein, especially peptones, which are present in pus or damaged tissue. Sulfanilamide, sulfapyridine, and sulfathiazole may produce mild, moderate, or profound intoxication and injury to the red cells, white cells, and bone marrow. They may cause skin rashes, fever, jaundice, or delirium, and the acetylated forms of the latter two may block the kidney

tubules and thus produce anuria. In a few cases of idiosyncrasy, these effects may take place promptly after administration, but usually they do not occur until the second week of administration. The toxic effects of the drugs generally stop promptly after their withdrawal.

On the basis of this knowledge, it was deemed essential to carry out a well-controlled study of drug treatment in severe accidental civilian wounds simulating war wounds. The plan called for ten research units in as many different cities, each unit to be fully equipped with clinical and laboratory facilities to care for and study cases of civilian accidental wounds, compound fractures, and burns simulating war casualties. All the wounds were to be treated basically by as complete a surgical débridement as possible. The débrided tissue was to be sent to the laboratory for a complete analysis of the bacterial flora. A limited number of different kinds of local and general treatment, with a proper series of controls, were to be employed in order to appraise, if possible, the newer chemotherapeutic agents in the prophylaxis and treatment of wound infection. Careful observations and records were to be made of the wound healing, and particularly of any evidence of infection. If infection developed, its nature and etiology were to be determined by further laboratory studies and the cause of the failure of the preventive measures was to be analyzed. A carefully prepared summary sheet was planned, to contain all the available data from the record of the case that might indicate what factors favor or minimize the development of infection. These data could then be transferred to punch cards for statistical analysis.

A careful estimate of the cost of such a study indicated that the chief expense would be the care of the patients and the setting up of the laboratories. It was thought that reliable data could be obtained on 1000 cases in three months' time at a total cost of $120,000. This sum seemed huge, but it was believed that the results of the study would save many lives and many dollars. With the government life-insurance cost of a fatality set at $10,000 it could not be considered an unwarranted expense. Assurance was given that the money would be forthcoming. Therefore the units were selected and the personnel chosen. The urgency of the situation seemed to be great because of England's expected downfall, but the plan had to be postponed because the mechanism for initiating medical research had not been clearly worked out. Eighteen months passed by before the plan was approved and the study could proceed. In the meanwhile many of the selected personnel of the units had been called to the colors, scientific apparatus was hard to obtain, and the rationing of gasoline and rubber cut down materially on the number and severity of civilian motor accidents. Pearl Harbor had received its catastrophic blow, and war was upon us before a solution of the problem had been reached.

Despite these obstacles, the plan of study was finally approved in all its essential features by the Committee on Medical Research. Seven units were

set up to study the prevention of infection in soft-part wounds, compound fractures, and burns, and two other units were organized to make observations on burns only.

During the period of delay, sulfadiazine had come into use. It was found to be less toxic than sulfanilamide, less nauseating than sulfapyridine, and less likely to block the kidneys than sulfathiazole. Its range of antibacterial activity was thought to be as wide as that of any of the other drugs, and it was therefore selected as the drug of choice for systemic administration. For local application equal parts of sulfanilamide and sulfadiazine powder were used, on the theory that the sulfanilamide in a concentration of 1000 mg. per 100 cc. would be active for several days before it was absorbed. The more slowly absorbable sulfadiazine would then continue to act over a long period of time, possibly ten or fourteen days.

No attempt was made to dictate the details of surgical procedure. The surgeons were directed to perform as complete a débridement of wounds as possible but were given full liberty to decide other details of treatment. In the units in which non-drug-treated controls were used, every care was taken that there should be no selection of controls or treated cases but that they should alternate regularly.

With regard to burns, there were strong advocates for three different methods of treatment. These were the tannic acid method, the vaseline compression-dressing method, and sulfadiazine in triethanolamine spray. A fourth experimental method was permitted each of the units.

Great pains were taken to make accurate observations and careful records so that it could be known just how the cases were handled. It was decided that all those having the care of patients should determine, as a group, whether infection had developed and whether it was trivial or serious. The criteria of infection included not only the bacteriologic findings but also the clinical evidence of inflammation; namely, redness, swelling, pain, fever, undue exudate, necrosis of tissue, or delay of wound healing. There may have been differences of opinion on individual cases, but on the whole the personal equation was fairly well balanced. As the responsible investigators of the different units gathered in Washington to talk over their experiences, it was obvious that all the units had had similar experiences and analogous results.

The summary sheets were completed as soon as possible after the healing of wounds had taken place. Preliminary reports were sent in on all compound fractures within two months, and changes were incorporated later in the record if infection or delayed bone healing supervened.

As the records came in, the data were transferred to punch cards and were then analyzed according to common factors. For the most part, it was possible to divide the cases into two or three groups within each category. For example, they were separated into those having maximum and minimum

gross contamination and similarly maximum and minimum tissue damage. Those operated on within three hours were compared with those operated on after three hours. With regard to wound closure, three groups were compared; namely, wounds left open, wounds partially closed, and wounds completely closed. A similar classification was applied to all the factors that it was thought might play a role in favoring or minimizing the incidence of infection. In each of these groups the number and percentage of trivial and serious infections were determined. As the summary sheets steadily accumulated in the central office, the number of cases in each group rapidly mounted, and it became necessary to apply the statistical formulas employed by biostatisticians to determine whether percentage differences are statistically significant or are due merely to chance. The investigators and their assistants met with the Subcommittee about every two months. At these conferences the results of the study were discussed, and agreement was reached on the trend of events and the conclusions to be drawn.

After six months, when it was found that the drug-treated cases were yielding no better results than the controls, it was decided that the local use of equal parts of sulfanilamide and sulfadiazine was not satisfactory. These drugs called forth profuse exudation, and the sulfadiazine often caked in the wound and acted as a foreign body for several weeks. The local treatment was therefore changed to sulfanilamide alone, and this procedure was followed for a second period of six months. This method also failed to show any benefit, so that in the third six months the local use of sulfonamide drugs was given up altogether. After eighteen months data had accumulated from almost 2200 cases, and these were carefully analyzed, with the following results.

GROSS RESULTS

In the whole study there were 2191 cases analyzed, of which 926 were soft-part wounds, 674 were compound fractures, and 591 were burns.[1] Roughly one sixth of the soft-part wounds, one fourth of the compound fractures, and almost one half of the burns became infected; in other words, they failed to heal without some evidence of bacterial invasion of the tissues. In analyzing these cases, attention was focused primarily on the results obtained with the sulfonamide drugs.

The term "infection" has been defined as the reaction of the tissues in and about the wound to the presence of micro-organisms or their poisons. Infections were graded as serious or trivial, the former being those that pro-

[1] The reader who desires to have access to the complete tabulated data is referred to the following published article: Meleney, F. L. A Statistical Analysis of a Study of the Prevention of Infection in Soft-Part Wounds, Compound Fractures, and Burns, with Special Reference to the Sulfonamides. *Surgery, Gynecology, and Obstetrics,* 80:263 (March, 1945).

duced general symptoms, further destroyed tissue, or materially prolonged the period of wound healing or hospitalization. The determination of the presence and severity of infection was the consensus of all the doctors in charge of the patient, and while there may have been a difference of opinion in borderline cases, the decisions must be considered reasonably accurate and consistent with the other evidence in the case. One may properly say that it is only the serious infections that deserve consideration, but it must be remembered that the line between serious and trivial is not distinct. It is of interest to note, however, that the statistically significant differences are for the most part in the "serious" group. The criteria of infection were based not on the finding of bacteria in the wound, either at the start or later in the course of wound healing, but on the clinical evidence of the response of the body to the presence of these organisms.

Soft-Part Wounds

There were 926 soft-part wounds, with an incidence of 6.4 per cent serious and 11.2 per cent trivial infections, a total of 17.6 per cent. This is three or four times as high a percentage as is found in clean operative wounds in any well-ordered hospital.

All the soft-part wounds were divided into either two or three subgroups to bring out the role played by some of the chief factors associated with infection. It was then found that there was a high incidence of infection in multiple wounds as compared with single wounds; in those with greater gross contamination as contrasted with those with lesser gross contamination; in those with greater tissue damage as against those with lesser tissue damage; in those operated on later than three hours as contrasted with those operated on within that period; in large versus small wounds; in those with incomplete as against those with complete débridement; in long versus short irrigation; and in partial closure of the wound as compared with complete closure or no closure. In most of these categories there has been statistical confirmation of what has been learned from clinical experience regarding the main factors concerned with the development of, or resistance to, infection. It was of interest that significant differences in the total percentage of infection were found in all but one of the factors mentioned above. These differences were either in the serious infections or in the trivial ones, and sometimes in both. All these groups required further breakdown, for other factors played important roles in each of these categories and might weight the figures one way or another so as to disturb the comparability of the main groups. For example, one might expect the group with prolonged irrigation to include a larger number of cases with maximum gross contamination than the group with a short period of irrigation. For this reason, groups had to be subdivided and cross-tabulated.

When all the soft-part wounds were divided into subgroups according to the method of treatment, without regard to the factors favoring infection, it was found that they fell into five principal categories. There were 322 cases that had neither general nor local sulfonamide; 153 cases received systemic sulfadiazine without any local drug; 187 cases received systemic sulfadiazine with local drug consisting of equal parts of sulfanilamide and sulfadiazine; 225 cases received systemic sulfadiazine and local sulfanilamide; and 39 were given miscellaneous types of sulfonamide treatment.

The results in the three main categories of drug treatment yielded no significant differences. The very small series of miscellaneous treatments showed a significantly smaller number of infections, but for various reasons these cases were not comparable with the others, and one cannot conclude that the miscellaneous drug treatments are any better than the three regularly employed. The similarity of the three main drug groups ran through all the cross-tabulations. It seemed to be of particular interest that the combined general and local use of drug was no better than the systemic administration alone. With no difference in results shown between the three methods, it seemed justifiable to pool the whole drug-treatment experience and consider it against the control experience. This comparison indicated that the control patients did significantly better than the treated patients, with 4.7 per cent serious and 7.8 per cent trivial infections, as compared with 7.3 per cent serious and 13.1 per cent trivial infections among the treated patients. But further breakdown showed that as a group the controls were somewhat less severely injured than the drug-treated cases. It was therefore necessary to cross-tabulate within these groups and to compare subgroups having the same common factors.

When the soft-part wounds were divided in such a way that the drug-treated cases and the controls could be compared within subgroups featuring certain factors thought to be of major importance with respect to the development of wound infection, it was found that in every category (with one close exception in the smallest group) the total percentage of infections was higher in the drug-treated cases than in the controls, and this generally held true for both serious and trivial infections.

Many more cross-tabulations were made, but they only confirmed the results revealed in the ones mentioned above. No matter how one looked at these results, one could find no evidence to support the thesis that the use of the sulfonamides lowered the incidence of infection in the accidental soft-part wounds covered in this study.

Of primary importance were, of course, the kind and degree of bacterial contamination. An attempt was made in one unit to make a quantitative determination of the number of organisms present, but this was not found feasible. It cannot be claimed that all the qualitative analyses of the bacterial flora were complete or that the rarer species of organisms were always

properly classified, but the positive findings of the four main groups of pathogens seemed reasonably accurate.

These four groups comprise the hemolytic streptococci, the coagulase-positive *Staphylococcus aureus,* the pathogenic aerobic gram-negative bacilli, and *Clostridium welchii.* The cases were divided in such a way as to show not only the occurrence of these bacteria in the débrided tissue but also the number of times the four groups of microbes persisted. Likewise it was noted in how many cases these organisms were found later when they were not found originally, suggesting either a secondary contamination or the persistence of organisms originally present in such small numbers that they were missed in the first cultures. Furthermore, the presence, persistence, and new appearance of these organisms were all correlated with drug treatment and the presence or absence of wound infection. It was found that the organisms disappeared as readily from the wounds of the control cases as from those receiving primary drug, and there is no indication whatsoever that the drugs primarily administered either locally or generally or both combined were able to eliminate these organisms from the wounds. This is just as true of the hemolytic streptococcus and *Cl. welchii,* which are ordinarily considered susceptible organisms, as it is of the coagulase-positive staphylococci and other micrococci and the pathogenic gram-negative aerobic bacilli, which are more resistant.

In considering the incidence of the different organisms during the course of the treatment, one was struck by three things. The first of these was the small proportion of cases in which pathogenic organisms were found in the original cultures of the débrided tissue, which later developed clinical infection. This is particularly striking with *Cl. welchii,* which was found in 138 cases, while infection developed in only 32, and in only 2 of these was there clinical gas gangrene. Second, there was a rather small proportion of cases in which the organisms found originally persisted in later cultures; for example, one fifth with the hemolytic streptococcus, two ninths with coagulase-positive *Staph. aureus,* one sixth with the pathogenic gram-negative bacilli, and one eleventh with *Cl. welchii.* These figures indicated not only the thoroughness of the removal of the organisms along with the débrided tissue but the ability of the body to take care of the organisms left behind. Third, although these organisms in some cases persisted in later cultures or appeared later for the first time, there was not always evidence of clinical infection. This clearly indicates that the criteria of infection were based not on the presence of pathogenic organisms in the cultures but on the clinical manifestations of their presence.

In spite of these far from satisfactory results with the sulfonamides in the prevention of infection in accidental wounds, there were only 4 deaths from infection in all these 926 cases. Three of these cases received primarily sulfadiazine systemically and sulfanilamide in the wound. The fourth was a

control. What does this signify? Perhaps the most plausible explanation is that the sulfonamides, while they were not able to prevent or minimize local infections, were able to prevent the general spread of infection to the rest of the body. Of these 4 fatal cases, 3 were among the drug-treated patients and 1 was among the controls, but the drug-treated patients all had serious injuries that may have contributed to the fatal outcome. When infection developed among the controls, the worst cases were treated with sulfonamide, as was done with the fatal case, so that it cannot be said with certainty that there would have been a higher incidence of sepsis and death among the controls if they had been carried through completely without drug.

The results of this study should not be interpreted as indicating that the sulfonamides do not have an important place in the treatment of infection. But their role is not prevention of local infection in the wound. It is probable that they prevent local infection from becoming general and causing sepsis and death. The problem of the development of local infection in these wounds still remains unsolved, and it will not be solved until some way can be found for the sulfonamides or some other agent to be effective in wounds in the presence of damaged tissue. Furthermore, the paramount objective is not simply the prevention of infection, but rather the attainment of the promptest healing that the degree of tissue loss permits. A wound that is in an unhealthy condition with respect to mechanical and physiological factors will not heal rapidly even though the local activity of bacteria is completely controlled. It must therefore be recognized that the prevention of bacterial action by chemotherapeutic agents is a highly desirable, but nonetheless a limited, objective. The final goal can only be attained through the application of surgical procedures designed to provide the best possible conditions for healthy wound repair.

Compound Fractures

The problems of compound fractures are two in number: the control of infection, and bone healing with subsequent muscle and joint function. This report, however, considered this matter only from the point of view of infection. The end-result desired in compound fractures is a normally functioning member, with firm bony union and freely contracting muscle around the bone and freely moving joints on either end of the fractured bone. This result is materially altered or delayed by infection within or around the bone or in the soft parts. Bone is less resistant to infection than are soft parts, and it is frequently cut off from its blood supply, either as a loose fragment or as the exposed end of the shaft that has been stripped of its periosteum. Infection may develop at once or within the first few days after the injury. It may start with the first efforts to obtain full motion of the part, or it may lie dormant for months after the wound has apparently

healed and then suddenly or gradually develop after some trauma or general lowering of resistance such as chilling or fatigue.

There were 674 compound fractures in this series, with 14.1 per cent serious and 11.4 per cent trivial infections, a total of 25.5 per cent. Whereas there were twice as many trivial as serious infections in soft-part wounds, the serious exceeded the trivial in compound fractures. This was to be expected, because when bone becomes infected wound healing is usually delayed and hospitalization prolonged. In many of the trivial cases only the soft parts were involved in the infection.

The principal factors concerned with the incidence of infection in compound fractures were found to be shock, greater gross contamination, greater tissue damage, and larger-sized wounds, all of which gave a significantly high percentage of serious infections. Other factors were apparently less important, but all groups required cross-tabulation to bring out their real significance. A surprising result was seen in the category of wound closure. The lowest percentage of serious infection was found in the wounds that were completely closed, whereas the percentage in those partly closed or left open was high. This finding was consistent throughout the course of the study, and it seemed to be statistically significant. But it was generally recognized that patients who had complete closure were, as a group, less seriously injured and were treated earlier than those whose wounds were left open. Another possible explanation for this is that when a compound fracture is left open or partly closed, the exposed bone, which is slow in being covered over with granulations, especially when there are separated fragments, is subject to prolonged exposure to the secondary contamination of organisms, which are thus more likely to gain a foothold.

When the compound fractures were divided according to the various forms of drug treatment, the results in the controls, representing 187 cases, did not differ significantly from those in the 487 cases receiving sulfonamide. In the controls there were 12.3 per cent serious and 12.8 per cent trivial infections, a total of 25.1 per cent, whereas in the drug-treated cases there were 14.8 per cent serious and 10.9 per cent trivial, a total of 25.7 per cent. Furthermore, the results with the three major methods of drug treatment mentioned above for soft-part wounds did not differ significantly from one another.

When comparisons were made between the controls and the drug-treated cases within the framework of the different factors that play a role in wound infection, only one statistically significant difference was found, and that favored the controls. What is more significant, in the groups in which the factors that favor wound infection were maximal, the figures for the drug-treated cases in all but one instance exceeded those for the controls. In the groups in which the factors that favor wound infection were minimal, the controls were higher except in the category of "small area."

When comparisons were made between the controls and the drug-treated cases by cross-tabulations that combined two major factors concerned with wound infection, it was found that in the two categories in which those factors were maximal the figures for the drug-treated cases were higher than the control figures, although the differences fell just short of statistical significance.

The evidence seemed clear that in those situations in which local wound infection was likely to develop, the sulfonamides as used in these civilian compound fractures failed to demonstrate any definite prophylactic value.

Of major importance in the development of infection in compound fractures is of course the presence of pathogenic bacteria. Cultures of all the débrided tissue revealed many species, and it will require a special study to determine the significance of all the organisms found.

The four main groups of pathogenic organisms have been considered, and the records have been divided in such a way as to show the number of cases in which the organisms originally found in the débrided tissue persisted in later cultures, and the number of cases in which they appeared as new cultures not originally found. All these data were correlated with the method of treatment in the three major categories of sulfonamide therapy and in the controls. It was found that the compound-fracture wounds were generally more highly contaminated with pathogenic organisms than were the soft-part wounds, and that a higher proportion persisted than in the wounds of the soft parts. A little over one quarter of the hemolytic streptococci, a little over one third of the coagulase-positive *Staph. aureus* strains, one sixth of the pathogenic aerobic gram-negative bacilli, and one seventh of the *Cl. welchii* persisted. Thus it was evident that the débridement of the wound and the defenses of the body cleared the wound of the great majority of the contaminating organisms, but this held true no more for the sulfonamide-treated patients than for the controls. Furthermore, the use of the sulfonamides did not prevent pathogenic organisms from appearing anew when they were not found in the débrided tissue, arising either from organisms present in such small numbers that they were not originally found or from organisms gaining a foothold as secondary contaminants. This held true of the wounds that were closed as well as of those that were left open.

In spite of the unsatisfactory record of the sulfonamides as preventives of local infection in these compound fractures, only 2 deaths from infection occurred in the whole series (0.3 per cent). In both cases the patients suffered injuries that were severe and played a part in the fatal outcome. Both patients had local and general sulfonamide treatment, and although they died, they did not have septicemia. When infection developed in the control cases, sulfadiazine was generally administered, and in these cases there were no deaths and no septicemia. It seems fair to conclude, therefore, that while the sulfonamides failed to prevent local infection in compound fractures,

they minimized the general spread of the local infection and may have reduced the number of deaths. But the problem of the prevention of local infection in compound fractures remains unsolved. Progress toward this objective must meet the same conditions as have already been discussed in relation to soft-part wounds, but the added complicating factor of bone injury must be considered as well.

BURNS

The problem of severe burns has five phases: shock, toxemia and nitrogen imbalance, infection, slough separation, and repair. Although these phases reach peaks of importance at different periods during the course of illness, they overlap to some extent. Infection has always been a serious problem in burns. Since the recent improvement in our understanding of the proper treatment for burn shock, infection has become of greater significance because of the large number of seriously burned persons who have survived the shock phase. Infection is a factor of importance from the moment of injury until the whole area is again covered with skin. Burns differ from other wounds in two vital respects: first, they are usually extensive but not deep, whereas other wounds are relatively deep but not extensive; second, it is not often possible or advisable to remove the dead tissue at the first surgical procedure as it is with other wounds. These two facts are significant from the point of view of infection because the contamination with organisms is greater and impossible to remove, and the medium that sustains their growth — that is, dead tissue — remains to favor their development. The bacteria causing infection in burns may be those residing normally in the hair follicles or sweat glands or those deposited on the surface subsequent to the burn. A superficial burn caused by a relatively low temperature applied for only a short duration may not kill all the organisms in the hair follicles. A deep burn caused by a higher temperature applied for a longer time probably kills all the bacteria in the skin in the central areas, but at the margin there must always be areas where the burn becomes superficial and the organisms present there remain viable and capable of growth.

In this study the clinical observers tried to maintain a uniform conception of the meaning of the terms "second-degree" or "third-degree" burns and "serious" or "trivial" infections. A second-degree burn was one in which the superficial epithelium was injured to the extent of blistering and any condition severer than that, short of complete destruction of the deepest epithelial elements, so that restoration could take place from residual epithelium. A third-degree burn was one in which all the epithelial elements over a given area were destroyed so that epithelial repair had to take place from the margin or from skin grafts. It is obvious that the degree of any burn cannot be determined when the patient arrives at the hospital, or indeed until the repair has taken place, or has failed to take place, spontaneously.

Furthermore, there cannot be a sharp line between a deep second-degree burn where repair takes place from scattered residual islands of epithelium and a superficial third-degree burn. In this study, burns that had any amount of third-degree areas were placed in this category and an attempt was made to estimate as nearly as possible the percentage of the body surface so involved. Similarly, in those that had only second-degree areas the percentage of body surface was approximately determined according to the scheme of Berkow.

Five hundred and ninety-one cases were analyzed, with serious infection observed in 21.8 per cent and trivial infection in 24.7 per cent, a total of 46.5 per cent.

It was found that the main factor in the incidence of infection in burns was the depth or degree of the burn. The cases were almost equally divided between those purely second-degree and those having some element of third degree, but the former group had only 8.1 per cent serious and 17.0 per cent trivial infections, while the latter had 35.5 per cent serious and 32.4 per cent trivial infections. Other major factors, as with soft-part wounds and compound fractures, were gross contamination, amount of tissue damage, extent of surface area, and shock.

When both the second-degree and third-degree groups were divided into those treated with some form of sulfonamide and those receiving no drug in any form, it was revealed that there were approximately the same number of drug-treated and non-drug-treated cases in each group. There was no evidence, however, that the use of drugs lowered the incidence of wound infection. The sulfonamide-treated cases were divided into three groups — general treatment alone, local treatment alone, and both combined — and each group was studied for the incidence of infection in second- and third-degree burns.

In this study no one method of local treatment stood out above the others, but taken as a whole the compression-dressing method was found to be superior to the eschar-forming treatments.

The bacteriology of burns covers a wide range if a careful analysis is made of the bacterial flora present in the loose skin removed from the burned area. Swab cultures are entirely inadequate, and even the cultures of all the tissue removed cannot reveal with certainty all the organisms present in any given case.

When the incidence, persistence, and later appearance of the four main groups of pathogenic organisms were studied, it was seen that a surprisingly large number of cases in which these organisms were originally present failed to develop infection. It was not surprising to note the large number of cases in which these organisms appeared as new cultures, since these cases were constantly subjected to secondary contamination from the patient's environment. Furthermore, organisms not originally cultured but present in certain areas of the wound may multiply prodigiously in the course of a

few days if not held in check by some effective antibacterial agent. It was obvious from the results obtained in these studies that the primary use of sulfonamide was not associated with the more thorough elimination of these organisms than in the control cases, nor did sulfonamide treatment prevent the later establishment of these bacteria in the wound.

During the first year of this study it was particularly evident that the anaerobes were more easily eliminated from these burns than were the aerobes. Apparently the surface of dead skin does not offer the same favorable environment for growth and invasion as do the muscular layers. It was therefore decided at the end of the first year to omit the anaerobic cultures on burns unless infection developed. No anaerobic cultures were taken in 204 of these burns. The incidence of anaerobes in the débrided tissue is therefore based on 287 cases, anaerobes being found in 261.

In spite of the apparently questionable value of the sulfonamides in the prevention of local infection in burns, there were only 3 deaths from infection in the whole series (0.5 per cent). These were all second- or third-degree burns with 50 per cent or more of the body surface involved. Two of these patients were primarily treated with general sulfadiazine and one of these with local sulfonamide as well; neither developed septicemia. The third had no general drug. Tannic acid had been used before admission, but after admission the local treatment was changed to vaseline gauze and compression. *Staph. aureus* septicemia developed on the fifth day and failed to respond to penicillin, although death did not occur until the twenty-fourth day.

This may be considered a low mortality from infection in burns. When the controls became seriously infected, sulfonamides were often used systemically, and we therefore have no control series carried through the whole course of treatment without a sulfonamide to compare with the mortality figures in the sulfonamide-treated patients.

The sulfonamides have apparently been useful in treating cases of infection in burns by reducing its spread from the original site. This appears to have been done successfully in some cases that were not originally treated with the drug. There is no indication from this study that these drugs should be used as prophylactic agents in cases of burns.

THE PROPHYLAXIS OF INFECTION WITH MICRO– CRYSTALS OF SULFATHIAZOLE AND PENICILLIN

The study of the prevention of infection in accidental wounds and burns was continued for one more year in four of the units in order to appraise the microcrystals of sulfathiazole and penicillin. Two new units were set up to appraise the value of penicillin in compound fractures, and six units continued the study of burns.

The combined data from all the units studying soft-part wounds and compound fractures indicated that the microcrystals of sulfathiazole gave a lower incidence of infection than other forms of sulfonamide, but the results were not significantly better than those in the non-drug-treated controls.

Penicillin, used by one unit in soft-part wounds, yielded a definitely lower figure than in the controls, but in this group the inhibitors of penicillin action — namely, the gram-negative bacilli — were strangely lacking, and it is questionable whether these cases were truly representative of battle casualties or of the general run of civilian accidents in which these intestinal organisms are so commonly found as "fellow travelers" with the pathogenic cocci.

Penicillin, still scarce when the study was begun, was released by the Committee on Chemotherapeutics and Other Agents only for the prevention of infection in compound fractures of the large, long bones. The two new units, together with the four already operating, accumulated and observed 109 cases. These studies were controlled by cases treated with microcrystals of sulfathiazole and other sulfonamides and cases without drug, but the combined data gave no clear indication that either penicillin or the microcrystals of sulfathiazole had the power to prevent infection in compound fractures.

INDICATED DIRECTION OF FURTHER RESEARCHES

Further studies must be carried out with penicillin, which is now available in larger quantities than when these experiments were made, but the weakness being shown by this agent in mixed infections, due to the fact that so many organisms commonly present in such lesions, as well as in accidental and war wounds, are able to inactivate the antibiotic, suggests that too great reliance must not be placed on it in this situation, unless some means can be found to nullify the penicillin inhibitors or unless some other antibiotic or chemical agent not so inhibited can be found. Certainly it can be stated without fear of contradiction that in the treatment of civilian accidental wounds or battle casualties no antibacterial agent now available can make up for the neglect of sound surgical principles. The prevention of infection depends on prompt operation as soon as possible after the injury is received, the complete removal of dead tissue, loose bone fragments, and foreign bodies, the maintenance of a good blood supply, and adequate rest for the injured part. The use of the sulfonamides and penicillin has unquestionably minimized the generalized spread of infection from the site of injury, but it has not significantly diminished the incidence of local infection.

In burns, the figures gathered indicated that secondary contamination

is of very great importance in the development of infection, and there is an urgent need for the discovery of some antibacterial agents that can be used locally to inhibit the growth of either primarily or secondarily contaminating pathogenic and nonpathogenic bacteria. The employment of chemical or surgical methods to achieve early removal of deeply burned skin gives promise of affording a significant lowering of the severity of late infections in burns.

EXPERIMENTAL WOUND HEALING

EDWARD L. HOWES

THE OBJECTIVES of the program for experimental research on wound healing conducted by the Committee on Medical Research can best be understood by reviewing the state of knowledge about the treatment of wounds at the beginning of the war. At the onset of the conflict, principles for the treatment of certain types of wounds were well established. For example, infection could be prevented in operative wounds by excluding bacteria. The skin was cleansed and painted with antiseptics, instruments were boiled, and the linen worn by the surgeon and nurses and used to drape the wound was sterilized by steam under pressure. This aseptic technic was found to be preferable to the methods of destroying bacteria after they had become deposited in the wound, as was originally practiced and abandoned by Lister. Likewise this method was unsuccessful in traumatic wounds received on the battlefield. They were usually heavily contaminated with bacteria before treatment was instituted, and attempts to destroy them by means of antiseptics were completely ineffective in preventing infection. There were many reasons for this. Some antiseptics would not destroy bacteria in the presence of blood; most of them would not kill bacteria located within bruised tissues or within extraneous material such as pieces of cloth, splinters of wood, or bits of dirt implanted in the tissues during wounding. If an antiseptic did kill bacteria, it often killed tissues as well or seriously interfered with the process of healing.

In 1898 a German surgeon, Friedrich, found that if he cut away damaged tissue containing bacteria within seven hours of inflicting a crushed wound in an experimental animal, infection did not occur. When the truth became apparent during World War I that antiseptics failed to prevent infection in battle wounds, the French surgeon Lemaître and the English surgeon Gray applied the method of Friedrich to a large number of wounds. The French termed the process "*débridement*," and this form of treatment rapidly became accepted as the most successful method of dealing with all wounds of violence, whether occurring in battle or through accidents of civilian life.

To say that débridement solved the problem, however, would be an unwarranted exaggeration. In civil practice greater success was obtained be-

cause the wound could be débrided early and careful rest of the injured part could be instituted. In warfare, on the other hand, few wounds could be treated early; often tissues were so crushed that all the injured flesh could not be cut away, and complete rest could not be instituted because the soldier had to be moved to the rear. Hence, the incidence of infection in war wounds still remained too high.

Infection in a wound is serious for two reasons: first, because it delays healing and increases deformity; and second, because it may spread from the wound to the blood stream and to other parts of the body, with fatal results. To help minimize the severity of infection developing in wounds, the Army issued an order during the last war that wounds were to be left open after having been débrided. This wise precaution saved lives and further reduced the incidence of infection arising in wounds as the result of the original injury, but a new complication was encountered. A great number of the wounds became recontaminated with bacteria entering them from the air or from the upper respiratory passage of the persons caring for them, and secondary infections developed. During World War I, Carrel and Dakin devised a scheme of continuously irrigating wounds with a chlorine solution to combat the established localized infection. Considerable controversy arose over the efficacy of this treatment, but until the onset of World War II the method continued to be employed in treating infected wounds, especially if dead tissue was present.

Another method was developed during the Spanish Civil War. Trueta put into large-scale practice a procedure popularized in this country by Winnett Orr of Kansas City. The wound was débrided early and kept open with gauze packing. An unpadded cast extending well above and below the site of injury was then applied to the injured member. This cast was left on for a long time until it became saturated with malodorous secretions. Many advantages were claimed for this method by the surgeons of the Loyalist armies, but the conditions of that war were such as to preclude the keeping of complete records. Although many of the claims for the Orr-Trueta method were not substantiated in World War II, two important ones were confirmed. The method provided a fair degree of immobilization of the injured part so that the soldier could be transported without severe aggravation of shock and blood loss, and recontamination of the wound by new bacterial flora was minimized because the cast was difficult to remove. As Walton Martin expressed it, Trueta's method was a return to the day of "laudable pus."

During the years immediately preceding World War II, sulfanilamide was introduced for the treatment of wounds, and a new era of wound treatment appeared to have arrived. Only a small number of cases were treated with sulfanilamide in the Spanish Civil War; but the British used the powder under casts in the retreat from Dunkirk (from May 29 to June 1, 1940)

and rather extensively in the subsequent bombings of London in August, September, and October 1940. The initial reports from our Army and Navy about the results of sulfonamide therapy of casualties at Pearl Harbor were glowingly optimistic. Accurate statistics had not been accumulated, however, and details of just how to use this new chemotherapy had not been carefully worked out. In addition, the general toxicity of the sulfonamides for cells and their interference with the process of wound healing had not been thoroughly studied, although investigations by Taylor of Indianapolis and Allen of Chicago were disquieting. The powders produced irritation of tissues, increased the oozing of blood into the wound, and caused swelling of tendons. Moreover, many species and resistant strains of bacteria were found to be immune to the action of sulfanilamide, so that new sulfonamide drugs, such as sulfapyridine, sulfathiazole, sulfadiazine, sulfamerazine, and sulfasuxidine, were one by one synthesized and introduced into practice in an effort to meet the shortcomings of sulfanilamide. Each new drug discovered widened the range of antibacterial activity but presented new problems of solubility, absorption, and tissue toxicity. It should be emphasized that most of the advocates of local sulfonamide treatment in wounds recognized the need for thorough surgical débridement as well.

This, then, was the state of our knowledge of how to treat wounds at the beginning of World War II. It seemed probable that infections would continue to develop in war wounds, because methods of prevention were only partially successful.

A better method of treating established infection in wounds was needed. The use of sulfonamides might help to prevent infection and would almost certainly help to control the spread of infection, once established, but much remained to be learned as to how best to employ these drugs. Finally, there was considerable interest in finding ways of increasing the rate of healing of all types of wounds.

There was only one satisfactory way of answering these questions; namely, to turn to the use of experimental animals in the laboratory and to devise satisfactory methods through which to obtain preliminary evaluation of new procedures. This task fell to the Subcommittee on Infected Wounds and Burns of the National Research Council under the chairmanship of Dr. Allan O. Whipple of Columbia University, and the Committee on Medical Research authorized and financed a number of research projects in this field.

RATE OF HEALING

At the Radiation Laboratories of the University of California, there was developed a new method of measuring the rate of healing of wounds by using substances that constantly give off radioactive particles. These substances could be injected intravenously, and their path throughout the body

could be traced by a Geiger counter, which ticks whenever an electrical particle is discharged into the field of the instrument. The ticks became more frequent as radioactive substance accumulated in the wound area under test. It was hoped that the rate of accumulation of elements required in wound healing would serve to measure the rate of repair.

Although nearly all elements can now be made radioactive, the method was limited for practical reasons to the use of phosphorus, sulfur, and strontium. The first two of these elements are concerned with the chemical activity and division of cells. Wounds were made in the skin and liver, and bones were fractured, in small laboratory animals. After the radio-active element was given, Geiger counts were taken over the wounds and over normal tissues of the same kind, treated with the same elements, at various intervals. In this way the amount of accumulation of phosphorus and sulfur in the healthy and wounded tissues during the healing could be compared.

In order to correlate activity of cells with differences in the accumulation of the radioactive substances during healing of the wounds, another unique method was employed to indicate how many cells were dividing. By adding dilute citric acid to the cells, the proportion of actively reproducing cells could be determined in a hemocytometer. As was expected, the proportion of reproducing cells increased after injury, whereas in uninjured tissue only the small number of cells required to replace worn-out ones were found. The reproductive response of cells from young animals was found to be greater than that of cells from old animals. In the healing of bone the process con-tinued longer than in wounds of the skin. Sulfur appeared to be more needed than phosphorus in the healing of skin wounds, whereas in bone the reverse was true.

Following the above basic observations an effort was made to hasten the rate of wound healing with various substances — with extracts made from the testicle, from the adrenal gland, and from yeast cells (biotin), and with chromatin obtained from the nuclei of liver cells. As so frequently happens in scientific research, the adjunct procedure, the mitotic count, furnished a clue that one of these substances might promote healing, while the change in the radioactivity patterns failed to demonstrate any significant influence on the part of any of the stimulants employed. The mitotic count rose sharply under the influence of chromatin extract, whereas the other extracts did not influence the rate of cellular division. The substance in chromatin that seemed to stimulate division was extremely unstable and completely disappeared from solutions after five days, and attempts to obtain stable preparations that might be tried out in man were unsuccessful.

Although this work raised some important fundamental questions, it offered little likelihood of meeting any practical aspects of the healing of war wounds, and it was therefore discontinued before definitive conclusions

had been reached. One of the little recognized casualties of the war was that suffered by research on fundamental scientific problems; matters having only long-term significance were constantly forced to give way to other investigations of a more immediately practical nature. The work on the fundamental biologic aspects of wound healing is an example of this type of casualty. It is hoped, however, that peacetime conditions will permit a resumption of study along this line, because much more knowledge is needed concerning the special chemical requirements of the healing wound. Further investigations with radioactive tracer substances and tissue extracts may yield information of great theoretical and practical significance.

HEALING OF BONE

A group of workers at the University of Minnesota investigated the rate of healing of bone by analyzing the content of minerals in the newly formed callus as compared to their percentage in uninjured bone. Holes measuring $\frac{3}{16}$ inch were bored in the humeri of anesthetized dogs. The injury was allowed to heal for twenty-one days, and a sample of the newly regenerated bone was then removed. Each sample was analyzed chemically for calcium, phosphorus, and nitrogen. Comparison was made of the chemical composition of regenerated bone in control animals and of that in animals to which sex hormones and other drugs had been given. None of these substances seemed to change the rate of bone healing. One leg of a rat was then paralyzed, and after atrophy occurred the mineral content of the bones of this leg was compared with that of the unparalyzed side. It was thus revealed that loss of ash content did not account for total weight loss of the bones in atrophy. Furthermore, the study showed that the female sex hormones, estradiol and estrogen, prevented bone atrophy in male rats better than did any other substance.

At New York University, two investigators studied the rate of healing of bones in rats fed with thyroxin, the hormone of the thyroid gland. Thyroxin had been found to accelerate the differentiation of the skulls of young rats. In the experiments the fibula was fractured and its rate of healing in animals receiving thyroxin and in controls was followed by x-ray and by microscopic examination of the callus after varying periods of time. Thyroxin did not accelerate the rate of healing, and the conclusion was reached that the amount of callus produced depended largely on the character of the fracture and its subsequent alignment.

INFLUENCE OF DRUGS APPLIED LOCALLY TO WOUNDS

As has already been mentioned, from the time when it was found that the sulfonamides would arrest the growth of bacteria within the body, efforts were made to develop new compounds capable of acting on more types of

bacteria and of overcoming the inactivation that the sulfonamides suffered in the presence of pus. A group of investigators at the University of Minnesota undertook the task of studying synergists, potentiators, and antagonists of the sulfonamides.

In the test tube, under certain conditions, these investigators found that guanidine hydrochloride and O-ethylisourea-hydrochloric acid synergized the action of sulfonamides against the staphylococcus and *Escherichia coli* but not against *Proteus vulgaris*. No other substances among the many chemical compounds examined were found to possess these properties. Attention was called to marfanil, a chemotherapeutic agent that was not neutralized by the natural inhibitors of the sulfonamide group. They also found that the concentration of most substances needed to be effective against bacteria usually interfered with the rate of wound healing. In these studies, the chemicals were applied to donor sites made on the backs of guinea pigs, and later rabbit ear wound preparations, as devised by Howes, were used. Several substances that had been introduced as stimulants of wound healing were found actually to delay it.

An extensive program of study was conducted at Columbia University. There were three objectives in this investigation: to learn how various substances, including the sulfonamides, would influence the rate of proliferation of cells of adult tissues growing in culture; to correlate the effect of these substances on the rate of wound healing; and to determine how best to apply substances to the wound.

A large number of substances were systematically examined. The first step was to determine the effect of a substance on the rate of proliferation of cells growing outside the body in a tissue culture. Next, the substance was applied to an open wound, healing without contraction, and the rate of repair was measured daily by means of photography and expressed in terms of an arbitrary standard index.

The search for the best method of applying the selected therapeutic agent to the wound, whether as wet dressings or dry powders or in an ointment vehicle, proved to be one of the most complicated phases of the problem. The duration of contact of any substance with the cells is governed by its mode of application. For example, continuous wet dressings, although they provide a constant concentration of the drug employed, tend to macerate the skin and increase its bacterial flora; yet the tissues beneath the skin must be kept moist in order to heal. Intermittent wet dressings expose the tissues to a concentration of therapeutic substances only during the time when the solution is drying and its concentration is constantly increasing. Vehicles provide a longer period of contact provided the therapeutic agent can escape. The concentration coming from the vehicle was found to depend not only on the amount placed in it but on the solubility of this substance and the solubility of the vehicle or its capacity to emulsify. Further-

more, only vehicles possessing no inherent toxicity could be employed.

Five types of vehicles or bases were studied: grease (petrolatum), grease and an emulsifying agent, water-soluble gels, soaps (stearic acid), and plastics. With the addition of the emulsifying agent to the grease, wound secretions could leach out the medicament. The further addition of water-soluble gels produced still greater diffusion.

Among the emulsifying agents tested for their effect on healing, the oxycholesterol esters, lecithin, and the sorbitol derivatives proved to be the ones that could be used most universally. The gels, with the exception of carbowax, were found to have the same limitations as wet dressings in that they washed away in the wound secretions and failed to retain the therapeutic agent at the wound surface. Each of the substances under test was applied to several different types of wound in experimental animals, and gross and microscopic examinations of the tissue reactions were performed.

One of the most significant conclusions obtained from studying the influence of many different drugs and compounds was that the rate of healing could not be stimulated. An optimal rate could be obtained in many ways, and certain medications could modify inhibiting conditions in the wound to bring about this optimal rate, but none of the drugs yielded a rate better than the optimal one, although many tested, including biotin and chlorophyll, were reputed to stimulate wound healing. Most substances, in fact, retarded the rate of healing. The sulfonamide ointments available at the beginning of the war were of poor quality. The fact that certain vehicles might interfere with healing was forgotten, and often the base employed was sufficiently injurious to the wound to offset any possible gain to be expected from the use of the sulfonamide drug. This work placed the selection of both drugs and vehicles for topical use on a sound objective basis.

The armed forces insisted that new agents for local therapy should maintain their physical form and medicinal properties at temperatures encountered in various parts of the world, ranging from the intense cold of the polar regions and the stratosphere to the heat of the tropics. In addition, all medications to be used on wounds had to be put through a process of sterilization. These requirements, while inconsistent with the circumstance that most of the medications long employed by the armed forces did not meet these specifications, provided a buffer against sudden changes in a period when every week seemed to bring forth a new alleged discovery. On the other hand, considerable time of investigators was spent in testing the toxicity of substances that would meet these temperature requirements instead of attacking more fundamental problems.

One of the interesting side issues of the experimental studies was the testing of substances reputed to aid wound healing. Many inadequately tested preparations were submitted to the Army and Navy for the treatment of casualties. During the emotional upheaval of war, drugs and con-

coctions came in with recommendations from all possible sources, and even some government officials were quick to lend support to a drug that they had been persuaded might save life or limb. Some remedies submitted had scientific background; others were backed only by romantic and mysterious tales, and still others were promoted simply because someone hoped he could make a profit. On the other hand, some interested persons and companies spent large sums without any thought of remuneration in an attempt to develop useful medications. Unfortunately, the greatest pressure was frequently encountered for acceptance of products with the least scientific merit. Actually every product recommended to the armed services was screen-tested, no matter how meagre the supporting evidence might be. Useful materials sometimes derive from unlikely sources, such as penicillin, a product of bread mold. Consequently, every suggested remedy was given tests on animals and against bacteria to determine its true worth.

As was to be expected, most of the substances submitted were without benefit. The indispensability of the laboratory animal was never more clearly displayed than in the testing of new agents before applying them in human therapy. Many of the materials tested would have been extremely harmful if used on human subjects. One preparation, for example, that came highly recommended from an official of a large city, when used as recommended not only did not speed healing of the rabbit's ear but actually ate a hole through the wounded portion.

Investigations of the sulfonamides were practically discontinued when penicillin was produced in quantities sufficient for military purposes. Studies of local toxicity, particularly in respect to wound healing and choice of vehicles, were focused on penicillin and the other antibiotics subsequently introduced. Sodium penicillin was found to be inferior to calcium penicillin for local use on wounds. Calcium penicillin of a high degree of purity proved to be almost completely devoid of injurious action on the fresh or healing wound. With penicillin, however, two new problems were encountered. It was highly unstable in certain vehicles, and it had no inhibiting action on the gram-negative bacilli that produced a penicillin-destroying enzyme, penicillinase. When used locally, therefore, penicillin needed some antibacterial substance that would attack the gram-negative bacilli. Obviously, this adjunct substance must also be devoid of toxicity to wounds. The metabolism of the gram-negative bacilli so closely resembles that of cells that nontoxic substances were difficult to find. However, at about the time the work of the Office of Scientific Research and Development was being terminated, Sulfamylon, or marfanil, parachlorophenol, and a new antibiotic, streptomycin, discovered by Waksman, were found to possess low toxicity for wounds and great activity against gram-negative bacilli. Thus it seemed probable that a suitable combination of antibacterial substances could be found.

The Columbia workers also devised a method of studying the therapy of infected wounds in animals. A square of skin and fascia was cut away from the back of the anesthetized rabbit, the underlying muscle was bruised, and the wound was contaminated with bacteria. Twenty-four hours later the wound became grossly infected. Various methods of treatment were tried. Pressure and infrequent dressings hastened resolution of the infection. Grease dressings sealed in pus more than did dry dressings. Nine antiseptics were employed as daily irrigations: Zephiran Chloride, carboxymethoxylamine, vioform, boric acid, oxyquinoline sulfate, parachlorophenol, phenol, alcohol, and iodine. None altered the progress of the infection in the slightest. Six sulfonamides were tried — sulfathiazole, sulfadiazine, 2-sulfanilamide carboxythiazole, Sulfamylon (marfanil), sulfasuxidine, and sulfaphthalidine — as powders, as ointments, and in solutions. All failed to hasten resolution of localized infection, although the first two, given parenterally, prevented the animals from dying of spreading infection.

Penicillin came the nearest to preventing infection from developing in these wounds. Once the gram-negative bacilli appeared in the wound, however, penicillin could no longer restrain the infection or hasten its resolution. By injecting locally a combination of Sulfamylon and streptomycin, infection could be prevented in wounds containing an excess of crushed tissues. This therapy was effective if the wound was treated immediately, but if an interval greater than three hours elapsed between receipt of the injury and the beginning of treatment, the effectiveness ceased. This avenue of investigation appears to deserve further study, since this might lead to more successful methods for preventing infections in accidental or battle wounds. Accordingly there is reason to hope that a combination of antibacterial substances may be discovered that will prevent infection from arising in wounds and cure the established infection when no dead tissue is present. It is also apparent that chemical methods must be devised to remove the dead tissue, on which bacteria thrive.

USE OF ACIDS AND ENZYMES

Beginning early in the war, intensive investigations of the role of acids and enzymes in liquefying slough in wounds were carried on, with particular attention to the selection of substances that would be effective under conditions existing in the wound and would not interfere with healing. By irrigating the wound for ten minutes each day with a mixture of acid pepsin and propylene glycol, followed by antibacterial agents, it seemed to be possible to hasten separation of slough and bring about the disappearance of pus.

A series of wounds in patients were treated with this method. Some resolved quickly, but those with anatomical configurations that did not allow

the acid to reach the slough were unaffected. The method is still under investigation, with particular reference to its possible usefulness in the treatment of burns.

OTHER METHODS

The influence of cooling on the healing of tissues and on infection was studied at Washington University, St. Louis. Refrigeration was being increasingly employed in the surgical treatment of gangrenous limbs, and exposure of limbs to extreme cold was suffered by airmen at high altitudes and by Navy personnel in subpolar temperatures.

As long as a temperature of 6° C. was maintained in the subcutaneous tissues of the dog, an inflammatory response was not initiated after an inoculation of streptococcus and the number of organisms remained relatively constant. Once cooling was discontinued, however, infection was much greater because of the edema that followed.

Wound healing was definitely delayed by low temperature. The process was studied both by measuring the tensile strength of the healing wound and by microscopic examination of the tissues. No reaction or healing occurred while the tissues were cooled, but after normal temperature had returned it was found that the lag period had been prolonged in proportion to the duration of the period of cooling.

Prolonged cooling also caused degeneration of the nerves of limbs of animals suspended in water at 6°C. for ninety-six hours, but without causing histologic changes in other tissues. In other words, nerve tissues appeared to be the most sensitive to the action of cold.

At Columbia University, the healing of surface injuries of the eyes was studied, and the effect of various treatments on the speed of the reparative process was noted. Superficial injuries of the eye resulting from burns or abrasions are likely to cause serious loss in efficiency and even permanent impairment of vision. Although the area of damage may be small, the patient is usually completely incapacitated during the period of healing.

Methods were devised for creating thermal and chemical burns and abrasions of the cornea of anesthetized rats similar to those met with in battle casualties and industrial accidents. Microscopic examinations were made of 3800 eyes, and the average rate of cell multiplication and the rate of covering of the wounded area were determined. With this background, the same injury was made in both eyes, and one was given the treatment to be investigated while the other was used as the control.

Practically all treatments slowed healing. Even the sulfonamides applied locally decreased the healing rate, although they might be needed to combat infection that had even greater propensity to stop healing. Penicillin could be used in bactericidal concentrations in the eye with no harmful effect on

healing. The study showed that some vehicles interfered with healing more than others and that some even harmed surrounding uninjured cells. It was demonstrated that needed drugs should be incorporated in vehicles that interfered least with healing.

Another phase of the study emphasized the importance of the form of medication. The size of particles of powders, for example, applied to the eye or incorporated in ophthalmic ointments affected the healing rate. Drugs that inhibited healing were much less harmful if the size of the particles was greatly reduced.

One of the more interesting and significant phases of the investigation dealt with local anesthetics. Cocaine needed to relieve pain delayed healing. Consequently, many other anesthetics were investigated. Some were found that did not appreciably delay healing.

The effect of special types of radiation on healing of the cornea and the relation of metabolism and age to the rate of generation were investigated. Substances that might accelerate healing were sought but not found.

Another project, with an indirect bearing on problems of wound healing, was the development of a new synthetic suture material at the Massachusetts Institute of Technology. The investigators had been for some time engaged in fundamental investigations on collagen, the principal constituent of the noncellular components of body tissues. It appeared to them that a useful contribution to the war effort would be the production of a surgical suture material composed of pure collagen. Such a suture might be expected to produce very little foreign reaction in the wound, and it was hoped that it would prove to possess many advantages over other absorbable sutures.

Ever since Lister, surgeons have employed sutures made from animal intestines in the closure of wounds. Being of animal origin, these "catgut sutures" were absorbed after a few days or weeks. Sheep intestines were cleaned and scraped until only the tough collagen fibers of the muscularis mucosa remained. The methods used by the investigators at the Massachusetts Institute of Technology were entirely different, being patterned after those employed to make rayon. The collagen was dissolved out of beef tendon by a series of chemical procedures, and a thick, viscous gel was obtained. This solution was forced through spinnerettes and into a fixative solution that solidified the collagen gel into soft threads, which after drying could be twisted and woven into many forms. By the same principle, membranes and tubes were fashioned from collagen.

Like catgut, this reconstituted collagen is rapidly absorbed after implantation in the body. Less inflammatory reaction develops around the site of implantation than around catgut, but the strand swells more and tends to have less tensile strength than catgut when imbedded in tissues. The problem of prolonging the maintenance of tensile strength of these sutures after implantation was a formidable one, but it had been partially solved from

the experimental point of view by the time the project ended. These sutures could be produced in many varied specifications, and each segment of a given strand passed specifications identical with those for all other segments. Determination of the practical usefulness of collagen sutures must await the carrying out of an extensive program of clinical testing. It is hoped that this work will prove to be a basis for substantial improvement in the quality and performance of surgical sutures.

CHAPTER IX

THE APPLICATION OF PENICILLIN TO SURGI-CAL PROBLEMS

JOHN WINSLOW HIRSHFELD

THE CARE of wounds and the control of their sequelae comprise the principal responsibility of the military surgeon. Owing to the circumstances under which war wounds are incurred, they are always contaminated by bacteria, either from the environment or, as in wounds of the intestine, from the victim's body. In the military surgeon's effort to restore the wounded serviceman to health, infection has been one of the most serious problems that he has had to combat.

The preliminary reports of British investigators indicated that penicillin might well revolutionize the treatment of many bacterial infections. It followed that the Medical Departments of both the Army and the Navy desired to determine with all possible speed the value of this new antibacterial agent in military medicine and surgery. Penicillin, however, was difficult and costly to make, and plants for its manufacture required much critical material, which a nation engaged in total war could not afford to risk if the venture might be without merit. It was essential to devise a means of testing the efficacy of penicillin as quickly as possible, not only to provide military surgeons with the information required for its intelligent use, but also to determine whether plans for its manufacture should be vastly expanded. In order to attain this objective, the entire supply of penicillin was concentrated in the hands of the Committee on Medical Research, which in turn delegated the active investigation of penicillin to the Committee on Chemotherapeutic and Other Agents of the National Research Council. The Subcommittee on Infected Wounds and Burns was asked to conduct a clinical evaluation of penicillin in surgical infections.

Fortunately, a number of research units had been assembled in leading hospitals throughout the country for the purpose of studying the prevention of wound infection in civilian accident cases. Each of these units was equipped with complete laboratory facilities and the special personnel needed for the sort of investigations that penicillin required. The study of this drug in surgical infections was therefore concentrated at first in these research units. The investigators and professional assistants in the several units assembled every two months in Washington and discussed methods of using

penicillin and the results obtained from it. In this way effective and co-ordinated work could be carried on simultaneously in many geographically separated centers. Important participating research units were those set up under Army auspices at the Bushnell General Hospital in Utah, particularly for the study of penicillin in infected war wounds.

The first task of the investigators was to learn something about the pharmacology of the new drug, and the original experiments of collaborating investigators were directed to this end. Penicillin was administered by various routes to a number of normal subjects, and its concentration in the blood, cerebrospinal fluid, and urine was determined at suitable intervals. The antibiotic was also given to patients with thoracic empyema, purulent arthritis, ascites, and pleural effusion. It was found that penicillin could be injected either intramuscularly or intravenously, and that when so administered it diffused throughout the normal body tissues and fluids, with the exception of the cerebrospinal fluid. It did not, however, diffuse into abscesses of long standing or into collections of pus in various body cavities, such as joints, or the pleural space. In order to sterilize pus in such locations, it appeared to be necessary to inject penicillin directly into the joints or the pleural cavity. It was found that the drug was excreted largely by the kidneys. This process took place with considerable speed, indicating that penicillin must be injected every two or three hours if adequate concentrations were to be maintained in the body fluids.

The fact that penicillin did not diffuse into the cerebrospinal fluid of normal persons made it necessary to inject it directly into the subarachnoid space in cases of meningitis. Subsequent studies proved that, while penicillin does not diffuse into the cerebrospinal fluid of normal subjects, it does so to a certain extent in persons with inflamed meninges. Whether this is sufficient to obviate the necessity of intrathecal therapy is still a controversial question. More recent investigations have revealed that penicillin applied directly to the brain in high concentrations is capable of causing convulsions, a factor that limits the amount that can be injected intrathecally. The preliminary studies seemed to show that penicillin could not be given by mouth, since it was destroyed by the hydrochloric acid of the stomach, but later work carried out when the supply of the drug became more abundant proved that this initial conclusion was not entirely correct. It is now established that about one fifth of the penicillin given by mouth is absorbed, provided it is given on an empty stomach.

The accumulation of this preliminary knowledge of the fate of penicillin following intravenous or intramuscular injection laid the foundations for a study of its value in the treatment of infections. Most surgical infections, such as infected compound fractures, peritonitis, and lung abscess, are not only caused by a mixture of bacteria but are complicated by the existence of many

other variable factors of importance in determining the course of the disease. Infected wounds in addition usually contain dead tissue or foreign bodies, which can only be dealt with by surgical methods. It was obvious that an attempt to apply penicillin directly to the treatment of these complicated surgical infections before more definite knowledge of its effect on specific bacteria in the body had been obtained might well result in a long period of investigation without any clear-cut results. To avoid this, it was decided to limit the preliminary investigation to the treatment of severe infections caused by a single organism.

Penicillin successfully inhibited the growth of *Staphylococcus aureus* in vitro. For this reason, initial emphasis was placed on the treatment of staphylococcal sepsis. Here was a disease with a mortality over a period of years of about 85 per cent, for which there had been no adequate therapy. The causative organism was known to be extremely susceptible to penicillin in the test tube, and the early work of the British investigators indicated that penicillin would cure certain staphylococcal infections in man. If it could be proved conclusively that this drug was a powerful therapeutic agent against the staphylococcus, expansion of facilities for its manufacture would be justified on this ground alone.

In the attack on this question, penicillin was distributed to a number of selected investigators with the stipulation that its use be confined to patients with severe staphylococcal infections or to cases of infection caused by other organisms ordinarily susceptible to the sulfonamides, such as beta-hemolytic streptococci or pneumococci, in which good results were not being obtained. These investigators were beset by every sort of pressure to release penicillin for the treatment of patients who were critically ill with almost every known disease. The drug was requested for the treatment of ulcerative colitis, arthritis, rheumatic fever, leukemia, cancer, and a host of other conditions. Had they yielded the precious supply, the penicillin would have been dissipated and very little information of value would have been obtained within the time required. By adhering rigidly to the criteria established by the Committee on Chemotherapeutic and Other Agents and treating only patients with severe staphylococcal sepsis or with pneumococcal and beta-hemolytic streptococcus infections that had not responded to the sulfonamides, the investigators were able to report conclusive results on many patients within a comparatively short period of time.

The results provided ample justification for expanding the production of penicillin with all possible speed. It was found that staphylococcal sepsis could be cured if infection of the heart valves was not present and if the patient was not too debilitated by the disease before therapy was begun. Those who early witnessed the dramatic effect of penicillin on many critically ill patients realized that it was certainly an antibacterial agent of strik-

ing value. Hemolytic streptococcus sepsis and pneumococcal pneumonia, which had failed to respond to therapy with the sulfonamides, likewise could often be cured by penicillin.

As the supply of penicillin became more abundant and the knowledge concerning its action became available, it was possible to expand the categories of infections on which it could be tried. Some of this work was done in civilian hospitals and some of it, because of the nature of the disease involved, was performed in military hospitals. As soon as the place of penicillin in the treatment of a given infection had been determined, active research on this disease was discontinued, provision was made to supply the drug only to patients with diseases in which its value had been established, and the supply of penicillin thus freed was diverted to the investigation of other conditions. This course was dictated by the scarcity of the drug and the enormous military requirements for it that soon arose.

Among the most interesting and spectacular of the surgical applications of penicillin growing out of these studies was the treatment of acute hematogenous osteomyelitis, chronic hematogenous osteomyelitis, chronic osteomyelitis arising from compound fractures, pulmonary and pleural suppuration, purulent arthritis, and peritonitis. These diseases will now be discussed in greater detail.

HEMATOGENOUS OSTEOMYELITIS

Acute Phase

This disease, caused by *Staphylococcus aureus,* has been one of the most disabling diseases of children. Attacking otherwise healthy children with startling suddenness, it has been responsible for a large number of deaths and an incredible amount of chronic illness. Even though a child recovered from the acute phase of the disease, it was apt to remain with him throughout life in the form of recurrent episodes of infection in different bones. The disease is primarily an infection of the blood stream with staphylococci, in which the organisms localize in one or more bones.

Before the advent of chemotherapy, treatment consisted of incising the soft parts down to the affected bone, drilling a number of holes in the bone, packing the wound with vaseline gauze, and immobilizing the limb in a plaster cast. This treatment did not always result in saving the patient's life, and convalescence was usually prolonged. It has now been established that penicillin administered in adequate doses early in the course of the disease, before much bone destruction has occurred, will effect a cure in a large number of cases without the necessity of surgical drainage. If the disease has progressed to the stage of extensive bone destruction and soft-tissue abscess formation, it is still possible to bring about a cure in the ma-

jority of cases. Under these circumstances, however, it may be necessary to resort to aspiration or drainage of soft-tissue abscesses and the local, as well as the systemic, administration of penicillin. Therapy in these more advanced cases must be prolonged further than if it had been begun early in the disease.

Although penicillin will not cure all cases of acute osteomyelitis, since some strains of staphylococci seem to be resistant to it, it has revolutionized the management of this disease. In the future, there will be fewer deaths from the acute infection and fewer patients will develop the chronic form of the disease.

CHRONIC PHASE

The success of penicillin in the treatment of acute hematogenous osteo-myelitis raised the question of whether its effect would be equally spectacular in the treatment of the disease in its chronic phase. In former years, many of the victims of the acute disease suffered frequent relapses, either in a bone that had previously been the site of disease or in an entirely new location. Furthermore, many patients had persistent draining sinuses, which in some cases caused only minor inconvenience but in others were crippling and incapacitating. Such a patient required frequent medical attention and often spent a good share of his life in a plaster cast. The accepted treatment, in addition to general supportive measures, consisted in surgical removal of the diseased bone, followed by packing of the wound with vaseline gauze, and immobilization of the bone by plaster casts. It was usually months before the wound healed and the patient could be up and about.

The application of penicillin to the treatment of chronic osteomyelitis demonstrated with considerable clarity an axiom that seems to apply to all chemotherapeutic agents; namely, that they will not sterilize dead bone, necrotic tissue, or foreign bodies or penetrate dense scar tissue. When these patients were treated with penicillin, drainage from the sinuses usually diminished and in many cases ceased. Patients who were greatly debilitated and febrile often became afebrile, their appetite improved, and they gained weight and strength. When treatment was stopped, however, a relapse occurred unless sequestra had been removed and dense areas of scar tissue subjected to surgical revision. If such surgical procedures were done under the protective influence of penicillin, many of these wounds remained closed. Furthermore, it was found that the surgical procedures that formerly would have been considered highly dangerous, such as excision of infected sequestra and scar tissue with immediate closure of the wound, could be done if penicillin was administered before and after surgery. In short, this drug was found to provide a means of improving both the local infection and the general condition of these patients, so that surgical measures designed to

remove dead bone and revise old scarred wounds could be safely performed with considerable hope of eradicating the disease.

Although the final estimation of end-results depends on long-time follow-up, it is already apparent that penicillin offers new hope to the victims of chronic osteomyelitis. Alone, it will not cure the disease, and it must be used in conjunction with surgery, but it improves the general condition of the patient sufficiently to make surgery possible, and its antibacterial power has enabled surgeons to concentrate on reparative procedures that would have been impossible before its advent. It is no longer necessary for patients with chronic infections of bone to spend endless months in a hospital waiting for spontaneous healing of a large infected wound.

CHRONIC OSTEOMYELITIS ARISING FROM COMPOUND FRACTURES

When a bone is broken at the site of an extensive soft-tissue wound, there results an extremely complicated and difficult surgical problem. It is necessary, if function of the extremity is to be restored, to keep the broken bones in proper relation to each other. If the bone is shattered, as is often the case with bullet or shell wounds, proper alignment is harder to maintain than if the bone had been broken into only two pieces. In order to restore function, it is necessary to cover the fragments of bone with soft tissue and to obtain healing without infection. Infection is so persistent in bone that prolonged if not permanent disability is apt to occur if such a wound becomes infected. The combination of infection, extensive loss of soft tissue, and fragmentation of the supporting bones of a limb is almost certain to cause prolonged illness, if not permanent disability, and frequently results in loss of the limb and sometimes in death.

Compound fractures incurred in war become infected with sufficient frequency to make their management a major problem. In order to learn whether penicillin could improve the treatment of these patients, several research projects were established in Army hospitals for intensive study of such cases.

Except for the extensive loss of soft tissue and fragmentation of the supporting bones of a limb, the problem was quite similar to that encountered in the management of chronic hematogenous osteomyelitis. The patients presented the picture of chronic sepsis. They were anemic, thin, demoralized, discouraged, and unable to eat. The pieces of dead bone, shell fragments, clothing, or other foreign bodies that were in these wounds correspond to the sequestra found in chronic osteomyelitis in preventing permanent healing. It was soon apparent that penicillin alone would not cure these patients, even though their wounds happened to be infected with bacteria susceptible to it. It was obvious, however, that penicillin, when administered systemati-

cally and locally, caused a great improvement both in the patients' general condition and in the condition of the wound. The patients' appetite improved, they seemed able to regenerate red blood cells, and the discharge from the wounds and the cellulitis about them diminished. If penicillin treatment was combined with adequate feeding and transfusions of blood, it was possible to transform an anemic, febrile, thin, septic patient with a wound draining considerable quantities of pus into a patient well prepared to withstand the effects of a surgical operation and likely to display good healing of his wound.

While penicillin alone would not cure these patients, it would do so when combined with adequate surgical revision of their wounds. Whereas the patient in his former state could not tolerate a major surgical procedure, in the improved state he could be permanently cured of his infection. Under the protection of penicillin, it was possible to remove foreign bodies and dead bone, to revise the soft parts so that the bone was covered, and to restore the normal contours of the limb, applying grafts of bone, muscle, and skin wherever necessary. Under this regime, thousands of men who might otherwise have lost their limbs or even their lives were restored to full health and economic self-sufficiency.

PULMONARY SUPPURATION

Lung abscess and bronchiectasis have always been difficult to treat. A lung abscess is essentially a cavity in the lung that communicates with a bronchus. The cavity is usually partially filled with pus, which the patient expectorates as it flows over into the communicating bronchus. Although some lung abscesses heal without surgical intervention, in the majority of cases it is necessary to resort to surgical drainage of the abscess cavity or to actual removal of the portion of the lung in which the abscess is situated.

Bronchiectasis is a disease in which the smaller bronchi become dilated so that secretions puddle in them and become purulent. Like patients with lung abscess, the victims of this disease usually raise large amounts of foul sputum. The disease differs from lung abscess in that it cannot be cured medically, because of the extensive anatomical changes present in the bronchi of the affected lung. Surgical removal of the diseased portion of the lung is the only means of restoring these patients to health.

The surgical management of lung abscess and bronchiectasis is complicated by the hazard of spreading infection to the uninvolved portions of the lungs and to the chest wall. If the patients do not cough up their infectious sputum, it lodges in the healthy portions of the lungs and sets up a new focus of infection. It is almost impossible during an operation on a patient who is forming large quantities of sputum and whose cough reflex is depressed by anesthesia to avoid soiling other portions of the lung. Not only does this

cause postoperative pneumonia, but when a portion of the lung is removed the pleural space that it has occupied is prone to become infected.

Penicillin was administered to a number of patients with lung abscess and bronchiectasis to see whether the activity of the infection could be reduced and the quantity of sputum diminished in preparation for operation. It was found that in the majority of cases, after several days of treatment, there was a remarkable decrease in the quantity of sputum. As was to be expected, this improvement was not permanent, because the anatomical factors responsible for the disease were not altered, and as soon as penicillin was stopped a relapse occurred. It was learned, however, that the drug made it possible to do curative surgery quite safely, not only because it controlled the sputum in many instances but also because, as in the case of chronic osteomyelitis, it caused an improvement in the patient's general condition. Frequently the temperature returned to normal and the appetite improved. There was less risk of postoperative pneumonia due to soiling of the unaffected lung, and in a large series of lobectomies and pneumonectomies the incidence of postoperative empyema was greatly decreased. Penicillin has therefore earned a place in the therapy of lung abscess and bronchiectasis, not as a curative agent, except in a few specially selected types of lung abscess, but as an aid to surgery.

PLEURAL SUPPURATION OR EMPYEMA

The power of penicillin to kill bacteria in the presence of pus is a property not possessed by the sulfonamides. Although it had not been possible for any of the sulfonamides to sterilize an abscess, it seemed that penicillin might be able to do so. Thoracic empyema in effect is a large abscess, and since it is frequently caused by bacteria susceptible to penicillin, it provided an opportunity to test this theory. A number of such cases were treated by frequent aspiration of pus and instillation of penicillin into the empyema. It was found that this therapy sterilized the empyema and that it could be cured without surgical drainage, provided that it was possible to aspirate all the pus, that a bronchopleural fistula was not present, and that the lung was able to expand to fill the space formerly occupied by the empyema. If, however, the pus contained large masses of fibrin that prevented its aspiration, or if one of the other anatomical conditions listed above was present, penicillin was of no avail, and the delay that such treatment caused merely prolonged convalescence and made the necessary surgery more complicated.

Ordinarily, it is necessary to aspirate the pus in empyema early in the course of the disease or to provide some form of suction drainage through a small tube. Later in the disease, as the pus becomes thicker, it is usually necessary to resort to resection of a rib to provide adequate drainage. Since penicillin can sterilize pus if used judiciously, it is capable of curing many empyemas in their formative stages. Often a few instillations of penicillin,

combined with aspiration of pus, are all that is necessary. If, however, this drug is abused by applying it to cases that are not suitable, not only will convalescence be prolonged but in many cases a chronic empyema will result. This is a condition in which infection of considerable duration causes the pleura covering the lung to become so thick that it prevents the lung from expanding to fill the space formerly occupied by the empyema. In order to cure such a lesion, it is necessary to move the chest wall in to meet the lung and thus obliterate the space. This can only be done by removing large portions of many ribs, a procedure that is not only time-consuming but permanently deforming.

Another procedure that has been made possible by the use of penicillin is so-called "decortication," or removal of the infected membrane and its contents, which allows the lung to re-expand and fill out the space formerly occupied by the abscess. By the time the war ended, decortication was being used with great success in military and civilian hospitals all over the world.

PURULENT ARTHRITIS

Purulent arthritis, or pus within a joint, presents a situation somewhat similar to that of thoracic empyema. The articular surfaces of bone are covered by cartilage that is peculiarly susceptible to infection. Pus destroys it rapidly, and once damaged it does not recover. Formerly, most patients with this type of infection were left with joints that were permanently stiff or at least had only a limited range of motion. All forms of surgical drainage were relatively ineffective. Not only was it difficult to drain a joint adequately, but motion caused so much pain that the joint became stiff from disuse in spite of adequate drainage.

The treatment of this disease by aspiration of pus and injection of penicillin directly into the infected joint has yielded results that are brilliant when judged by former standards. If the causative bacteria are susceptible to penicillin, the joint often returns to normal after a few days of treatment. This is true, of course, only if treatment is undertaken early in the disease, before irreparable damage has been done to the articular surfaces. If treatment is begun only at a later stage, after irremediable damage has been done to the joint surfaces, it is possible actually to excise the articular ends of the bones and to cause the newly exposed bone ends to become united to each other. This results in a stiff joint, but the patient is freed from the disabling consequences of prolonged infection.

PERITONITIS

Usually peritonitis resulting from the escape of intestinal contents is due to a mixture of bacteria, most of which are not susceptible to penicillin. When this drug was scarce, it did not seem advisable to employ it for the treatment

of this condition, because it was known that so many of the causative bacteria were not affected by it. It is logical to assume, however, that a patient infected with several types of bacteria would have a better chance of survival if one or two types could be eliminated by chemotherapy than if he had to rely entirely on his natural defenses. When, therefore, the supply of penicillin became abundant enough, a number of patients with peritonitis were treated with it.

Unfortunately, the results have not been very clear-cut. First of all, the course of the disease is unpredictable. There are not the reliable criteria for predicting its severity that exist in a disease such as pneumonia. Second, many of the bacteria that are not susceptible to penicillin are capable of elaborating enzymes that destroy it. This means that in the presence of such bacteria penicillin may be neutralized and therefore may not even be effective against susceptible strains. The entire problem of penicillin in the treatment of peritonitis requires more study, especially in regard to its value alone in very large doses or in conjunction with other antibiotics, such as streptomycin, which affect bacteria not susceptible to penicillin. Even though penicillin may not be of highly specific value against peritonitis itself, its use in such patients may be justified on the ground that it helps to prevent the pneumonia that frequently occurs in association with peritonitis.

SUMMARY

The experience gained in these investigations has demonstrated that penicillin is a powerful antibacterial agent against certain types of organism. There are, however, many kinds of bacteria that not only are unaffected by the drug but possess the power of destroying it. Penicillin gives its most spectacular results in the treatment of infections such as bacteremia, septicemia, and cellulitis. When localized abscess has formed, systemic therapy usually will not suffice, because penicillin does not diffuse into the abscess. If the abscess is accessible and the drug can be injected directly into it, penicillin will sterilize it, for, unlike the sulfonamides, it has the power to kill bacteria in pus.

Penicillin conforms to the general rule applying to all chemotherapeutic agents; namely, that they will not sterilize wounds harboring foreign bodies, necrotic tissue, dead bone, or dense scar tissue containing small abscesses. It will, however, under such circumstances cause a remarkable improvement in the general condition of the patient. This improvement makes it possible to withstand surgical procedures that would have been hazardous or fatal for a critically ill patient. This property of penicillin has made it possible to extend the opportunity of curative surgery to many patients who would not otherwise have enjoyed its benefits and has shortened the illness of many others.

The striking success that penicillin has had in the treatment of diseases caused by bacteria susceptible to its action has demonstrated the tremendous therapeutic possibilities of the antibacterial agents in general. There is ample justification for the expenditure of funds and energy in the search for other antibiotics that will be effective against bacteria not responsive to penicillin. It is reasonable to predict that the time is not too distant when an antibiotic will be available for almost every type of bacteria. It must be remembered, however, that experience to date has indicated that in spite of phenomenal antibacterial power, these agents are helpless when certain anatomical conditions prevail. There is as yet no evidence that any chemotherapeutic agent will permit the violation of sound surgical principles in the treatment of infections.

ORTHOPEDIC PROBLEMS AND PROSTHETICS

GUY A. CALDWELL

THE HIGH percentage of extremity injuries among combat casualties, many resulting in amputations, justified extensive investigation of related problems. Among these were the control or elimination of infections of the bones and joints; measures to ensure and hasten bony union of fractures; improved appliances, such as plates and screws, for the internal fixation of fragments and bone grafts; and the production of more comfortable and efficient artificial limbs for amputees.

Acute infections of the bones developed as a result of contamination of the associated wounds of the soft parts, and the infection became chronic when it had persisted long enough for the bacteria to gain a firm foothold in the bone. Whenever the wound of the soft parts that communicated with the fracture could be so treated that primary or early healing occurred, acute or chronic bone infection (osteomyelitis) did not occur. Fortunately, it was possible in many combat areas to organize the transport of the wounded so well that the lapse of time from infliction of the wound until definitive treatment and operation could be undertaken by a competent surgeon was reduced to a minimum. Plasma and blood were available to combat shock and hemorrhage. The presence of capable anesthetists and surgeons made it possible to perform adequate débridements and to remove foreign bodies, loose bone fragments, and infectious material from the depths of the wound. Trained assistants were on hand to apply large sterile dressings with gentle pressure and to affix splints or plaster to immobilize and rest the damaged structures.

These measures were found to be the major factors that governed the control of infection and early healing of the soft parts overlying the fracture. The use of sulfonamide drugs or penicillin locally or systemically appeared to be a useful adjunct to the foregoing procedures, but could in no way replace them. Most of these measures were verified by early experimental and clinical investigations before they were put into routine use in the theaters of war; detailed reports of these studies are made elsewhere.

While large numbers of those who had compound fractures were thus rescued from the delays and dangers incident to chronic osteomyelitis, many were not so fortunate, and chronic bone infections continued to be a major

problem in the later care of the wounded. Investigation of some of the basic factors relating to persistence of localized bone infection was therefore undertaken, and the following facts were demonstrated.

In the first place, most chronic infections are of a mixed type, part of the organisms being susceptible to penicillin or the sulfonamides and others being resistant to them. The latter could not be eliminated by systemic or local applications of known agents that could be used safely, and yet were capable of prolonging a low-grade chronic infection.

Secondly, the blood supply to the involved area of bone is greatly diminished, as shown by review of histologic sections. The number and size of the vessels entering the bone through dense overlying scar tissue are much less than those seen in normal bone covering; large nutrient vessels are frequently destroyed; and arterioles within the bone adjacent to the infected focus are found to have thickened walls and narrowed lumens, and the number of these is greatly reduced. Obviously, infectious organisms cannot be overcome and new bone constructed to repair a fracture or fill a bone defect when the local blood supply is seriously impaired.

These observations indicated that measures aimed at eliminating chronic localized bone infections must be directed primarily to improving the quantity and quality of the blood delivered at the site of infection. Controlled studies were made of a number of cases of chronic bone infection, and the following procedures appeared to be most helpful.

An increased quantity of blood can be made available in the bone adjacent to the chronic infection by certain physiotherapeutic measures carried out for some time prior to operation. These consist in raising and lowering of the limb, repeated contractions of the muscles, heat and massage (avoiding the immediate vicinity of the wound), and sympathectomy or sympathetic-nerve block. When the circulation of the entire extremity has thus been improved, the following surgical measures can be undertaken: excision of scar tissue and coverage of the bone with healthy muscle and skin; removal of the dense, sclerotic, avascular bone that forms the walls of the infected cavity; establishment of contact between healthy, bleeding bone and healthy, bleeding soft tissues; and closure of soft parts or the growing of full-thickness skin over the old infected area. The last of these steps was accomplished without tension, even if some other portion of the limb had to be robbed of its covering.

Improvement of the quality of the blood was begun some days or weeks in advance of the operative procedure with general hygienic, dietary, and medicinal measures to improve the general nutrition and blood picture. For several days prior to operation, doses of 20,000 to 30,000 units of penicillin were given every three hours. This dosage was continued for two or three weeks postoperatively. Transfusions were used liberally before, during, and after operation.

Under such a regime, primary or early soft-tissue healing (in ten to fourteen days) was obtained in 60 per cent of unselected cases of chronic osteomyelitis. Within a few weeks after healing of the soft parts over fractures with nonunion, it was usually possible to do a bone graft with little risk of recurrence of the infection.

Important experimental work was done on the bones of dogs and rabbits to determine the comparative merits of cancellous and cortical bone as grafting materials. The grafts were implanted in defects created in the tibia and the radius, in the spine, and across the knee-joint line after excision of the joint. Roentgenograms of the grafts and microscopic sections of the tissues were studied in each group of experiments. It was found that cancellous bone contributed to the growth of new bone and was therefore superior as a grafting material to cortical bone, which produced new bone only through the cells on its surface. Furthermore, because of the porous structure of cancellous bone it more readily became a part of the healing process. The greater part of a cortical bone graft must be absorbed and replaced by the surrounding bone. Experimentation and clinical experience show that cancellous bone is particularly useful in the fusion of joints and in defects and ununited fractures near the ends of the long bones. In ununited fractures of the shafts of the femur and tibia, where strength is required for weight-bearing, cortical bone is probably superior. In these cases it may be used together with cancellous bone chips.

Bone grafts have also been employed in compound fractures in the presence of infection. Their clinical use in such fractures shows that cancellous bone is superior for this purpose, since cortical bone does not survive in the presence of infection. A good surgical technic and the use of penicillin are necessary adjuncts to bone grafting in open and infected areas.

Experiments were conducted by other investigators on the rate of healing of fractures in animals when acetylthyroxin was administered. Simple fractures of the fibula in rats were produced by manual pressure. The rate of repair was followed by roentgenograms taken at regular intervals and also by microscopic sections made from animals sacrificed at different stages. The results of the investigation were essentially negative, and the investigators believe that acetylthyroxin is clinically useless in the treatment of fractures.

Other workers made an extensive study of the effect of choline on the metabolism of bone, with special reference to fracture repair. Periodic x-ray examination of bone-healing processes following fractures of the tibias of rats showed that these were not significantly accelerated by choline supplements. Furthermore, these supplements did not materially alter the progress of the atrophy of bone and muscle following unilateral section of the cords of the brachial plexus.

Frequent necessity for the use of metal plates and screws for internal fixation of bone fragments and grafts and conflicting reports on the suitability of

various metals and alloys for these purposes led to experiments aimed at determining their relative resistance to corrosion by body fluids. Vitallium, tantalum, and steel alloys were successively tested in five different media — physiological saline solution, dog serum alone, serum containing sulfanila-mide, serum inoculated with *Staphylococcus aureus,* and serum at pH 5, phosphate-buffered. Some of the significant conclusions reached were that in vitro RKA_2 (resistal KA_2 steel) showed less weight loss than the other ma-terials tested; in no case were there gross losses in weight or evident surface corrosion; RKA_2 plates were more rigid than any other; and bent or strained plates corroded more rapidly than unstrained plates.

In connection with prosthetics, experimentation was carried out by an investigator who improvised a prosthesis to replace the amputated foreleg of a dog, attaching it by skeletal fixation directly to the bone. The healing was satisfactory, and the appliance was used and well tolerated for more than a year. It was then observed by x-ray that some of the nuts holding the ap-pliance in place had loosened. In spite of this, the animal gave no evidence of having pain and continued to use the appliance successfully for another year.

Another animal on which a similar prosthesis had been fixed was sacrificed and the gross specimen and histologic sections were studied. The gross specimen showed excellent tissue about the prosthesis in the strong bony attachment; in fact, bone had grown around the prosthesis in several places. The histologic sections revealed that motion of the prosthesis attached to the bone had resulted in bone resorption. Efforts were made to conduct a similar test on a patient, but a suitable case was not available.

Because of the large numbers of amputees in Army hospitals and the universal lack of standardization throughout the artificial-limb industry, the Surgeon General of the Army decided that certain standard artificial limbs should be selected that would incorporate the best features available. To this end a meeting was called in Chicago on January 30, 1945, of members of the Panel on Amputations of the National Research Council, representatives of the Army, Navy, Veterans Administration, and Bureau of Standards, arti-ficial-limb manufacturers, and scientists from various related fields. Require-ments and specifications were discussed, and plans were formulated to supply a standard artificial leg to the Army hospitals. This consisted of a standard Hanger cast-aluminum knee joint with brake and hip control adjustment for above-knee amputees and a standard cast-aluminum ankle assembly. Both these joint assemblies could be bonded to metal, fiber, or plastics used in the construction of other parts of the limb, and both joints could be pro-duced in mass quantities. The England General Hospital in Atlantic City was designated to serve as a test center for plastic limbs and the McGuire General Hospital in Richmond, Virginia, for metal limbs. At the latter insti-tution, a plan to simplify modification of the metal sockets, which is neces-

sary from time to time because of the shrinkage of above-knee stumps, was put in operation.

Subsequently, in March 1945, the Committee on Prosthetic Devices met and planned both temporary and long-range programs to procure the best prosthesis available to meet the present emergency and to investigate ways and means of manufacturing still better appliances.

Pursuit of these aims required analytical studies of the mechanical behavior of normal and artificial limbs and a review of the mechanical features of existing prostheses. With the information thus obtained the Committee hoped to initiate research to simplify, improve, and standardize artificial legs and arms. This work was to include studies of materials and methods used to fabricate special structures and mechanisms, studies of the art of fitting and adapting the appliances to the requirements of amputees, and the problem of training the patient to use his prosthesis.

During the progress of its investigations the Committee was assisted by orthopedic surgeons, limb manufacturers, and certain patients who were using artificial limbs. As new devices and mechanisms were developed prototype models were sent to the various amputation centers and tested. When, in the judgment of the Committee, a satisfactory model had been developed, further consideration was given to the engineering problem of its mass production.

When the Committee reviewed the research problems connected with developing better prosthetic appliances, it found that they extended into many different fields. Accordingly, contracts for certain problems of mechanics and fabrication were given to an aircraft company, others to a tire and rubber manufacturer, a business machine corporation, and a plywood manufacturer, and still others to certain universities and research institutes.

In addition to the projects thus set up by the Committee on Prosthetic Devices, funds were appropriated and plans approved in September 1945 for additional investigations to be carried out in the seven amputation centers that had been established by the Surgeon General.

At a joint meeting of all the interested agencies in January 1946, reports of progress were made. These showed that the following accomplishments had been realized:

(1) There was devised an attachment to prostheses for amputations below the elbow that provided turning movements for the wrist and hand. This was tested and found to be so satisfactory that a contract for one thousand attachments was placed for the Army.

(2) For above-elbow amputees, an improved elbow joint was developed that could be locked in any position and in addition provided for pronation and supination of the forearm. This device was perfected and received clinical trials.

(3) A metal cable for control of the hook and hand of an upper-extremity

prosthesis was developed. It is similar to those used in airplanes and eliminates most of the friction and wear connected with use of the hook or hand. It has been adopted as standard equipment by all Army amputation centers.

(4) An improved hook for upper-extremity prostheses was made of an aluminum alloy. It weighs only a third as much as the old standard stub hook.

(5) The tire and rubber company completed models of a four-way ankle joint, made by a simplified process of bonding rubber to metal.

Contributions from the Army research program include the following:

(1) Bakelite thermo-setting plastic for making buckets for below-knee stumps. This material is resistant to all body secretions, is virtually indestructible, and can be used by inexperienced workers. It has been adopted by all Army amputation centers.

(2) Improvement of the multiple-acting elbow joint to permit complete flexion when used on contracted, short below-elbow stumps.

(3) A polycentric elbow joint to increase range and ease of motion.

(4) A wheeler knee joint so constructed that it does not buckle under weight-bearing.

(5) An ankle joint in which an adjustable tension spring is used. This eliminates the necessity for rubber bumpers.

(6) A satisfactory plastic glove to cover artificial hands.

THE PROBLEM OF GAS GANGRENE

WILLIAM A. ALTEMEIER AND W. L. FURSTE

G̲AS GANGRENE and tetanus are the most dreaded infections developing in wounds of violence. Although tetanus has been controlled by effective prophylaxis, the incidence and mortality of gas gangrene have remained essentially unchanged.

Gas gangrene has been recognized for many years, chiefly in association with war wounds, and many of the great names in surgery have been associated with its history. Hippocrates reported vividly a case of gangrene that apparently was of this type. He wrote:

Criton of Thasa commenced to experience pain in his foot, in his great toe. He had a slight chill, some nausea, and then a little fever; he became delirious during the night. On the second day there was swelling of the entire foot and over the whole ankle, which was a little red and tender; there were present tiny black blebs and he had a great fever. The sick one was completely out of his head. There were frequent evacuations of bilious matter. He died the second day after the onset of the illness.

A long period ensued during which the disease was apparently not recognized, and Avicenna, Guy de Chauliac, Giovanni da Vigo, and Ambroise Paré did not refer to it. In 1745, Quesnay attributed the first exact observations on gas gangrene to La Peyronnie, who spoke of "the subcutaneous emphysema, the erysipelatous color of the skin, and the rapidity of death." Later Dupuytren described a condition of "spontaneous emphysema" occurring in trauma and resulting in rapid decomposition. Fabricus de Hilden mentioned gas gangrene in 1746 and expressed his belief that "the principal cause of this terrible ill is some venomous humor which Nature has driven into these people."

Early in the nineteenth century, Larrey, the leading surgeon of the Napoleonic Wars, spoke of the rapid progress of traumatic gangrene, which spread from the injured limb in a few hours and was often fatal in less than ten hours. Boyer (1814), Velpeau (1829), Malgaigne (1834), and Martin de Bazas (1836) stressed its occurrence as a complication of fractured limbs and recognized its grave significance. Chassaignac (1849) indicated that certain gangrenes with emphysema seemed to have "a poison far in excess of the

mechanical injury," and Billroth considered the cause of gas gangrene to be the decomposition of the mortified elements. During the Crimean War, Pirogov and Salleron wrote of this condition, and in the Franco-Prussian War it was reported by Wyatt, Fréry, and Passow.

With the coming of the Bacteriological Era, the causal organisms of the disease were established by various workers. Pasteur discovered *Clostridium septicum* (*Vibrion septique*), Novy discovered *Cl. oedematiens,* and Welch discovered *Cl. welchii.*

There is evidence that the incidence of gas gangrene has been significantly increased in recent years. In the wars immediately preceding World War I, approximately 80 per cent of the wounds were caused by rifle bullets, and serious infections such as gas gangrene were infrequent. In modern war, however, high-explosive shells and bombs produced a high percentage of the wounds, which were different from the average wounds of preceding wars. When expectant treatment was applied to such wounds in the beginning of World War I, the results were appalling and gas gangrene was frequent. It soon became evident that the devitalized tissue in wounds must be removed to prevent this serious infective complication, and the effectiveness of operative cleansing of wounds by débridement was clearly demonstrated. Among the 224,080 men of the American Expeditionary Force who were wounded in France, there were 128,265 with wounds of the soft parts, with an incidence of gas gangrene of 1.1 per cent, and 25,272 with compound fractures, with an incidence of gas gangrene of 5.3 per cent. The death rate among those who developed gas gangrene in soft-tissue wounds was 48.5 per cent, and in compound fractures it was 44.6 per cent. In the more recent Ethiopian and Spanish wars, gas gangrene was relatively infrequent, but with the advent of active fighting in Europe in 1939 and 1940, the incidence of this complication in wounds again varied between 0.7 per cent and 1.8 per cent, with a mortality of approximately 50 per cent.

At the threshold of America's entrance into World War II, an incidence of gas gangrene of 0.5 to 2.0 per cent of all casualties sustained in combat or in the bombing of large cities was expected. Anticipating this, surgeons were confronted with these considerations:

(1) Very little was known of the factors determining the production of clostridial infections in wounds, although numerous studies revealed the incidence of such contamination to be 25 to 40 per cent.

(2) No effective method of preventing gas gangrene other than surgery was available.

(3) Considerable confusion had developed in the minds of many surgeons as to proper terminology, adequate means of diagnosis, and pathological interpretation, since gas gangrene was a clinical entity with numerous clinical manifestations. As a result, diagnosis was frequently delayed until the infection became so far advanced that amputation and often death resulted.

(4) Relatively little was understood regarding the alterations in physiological chemistry produced by these infections.

(5) The treatment of established gas gangrene presented many problems. Although the importance of adequate surgery was recognized, not only in prevention but also in control, there was general confusion and disagreement as to the therapeutic value and limitations of serotherapy, chemotherapy, and roentgen-ray irradiation.

(6) The mortality of established gas gangrene had remained high, being approximately 50 per cent, because of difficulties in diagnosis, lack of understanding of the nature of the infection, and confusion regarding methods of treatment.

In an attempt to solve as many of these problems as possible, the Committee on Medical Research established projects at the University of Rochester School of Medicine and at the University of Cincinnati College of Medicine.

In Rochester, investigators had been studying the effect of roentgen-ray therapy and oxygen on gas gangrene in dogs, and the contract with the Office of Scientific Research and Development permitted them to continue their investigations. In one series of experiments roentgen-ray irradiation of 25 dogs with infections of less than standard severity resulted in the survival of 36 per cent, as compared with 20 per cent in a similar untreated group of 25 dogs used as controls. Further study, however, showed that when severer or "standard" infections were produced with *Cl. welchii,* the rate of survival was almost identical in the group treated with roentgen-ray therapy (16 per cent) and in the control group (15 per cent). It was concluded that roentgen-ray therapy was possibly of limited value in the milder forms of gas gangrene, but that this value became imperceptible in the severer infections.

The investigators then turned to an intensive study of the value of the new chemotherapeutic agents and antitoxin in the prophylaxis and therapeusis of experimental gas gangrene. Infection was produced in dogs by injecting virulent cultures of *Cl. welchii, Cl. septicum, Cl. novyi,* and *Cl. sordellii,* singly or in combination, with a small needle deep into the muscles of the thigh through a previously prepared area of skin on the right hind leg. The combined clostridial inocula also contained a strain of *Staphylococcus aureus.* The amount was adjusted to cause death in a high percentage of control animals without producing an overwhelming infection, so that the effect of therapeutic agents would be more apparent.

With this technic, it was found that sulfadiazine was markedly efficacious as a prophylactic agent against pure or mixed infections produced by *Cl. welchii, Cl. septicum,* and *Cl. sordellii* and was far superior to roentgen-ray therapy (Table I). None of the sulfonamides were effective against the disease when it was produced by *Cl. novyi.* In these prophylactic studies

the first dose of the sulfonamides was administered intravenously just prior to bacterial inoculation, and maintenance doses were given orally or intravenously or both for three days.

TABLE I

Percentage of Ten-Day Survivals in Prophylactic Studies
(25 Animals per Group)

Prophylactic Agents	Cl. welchii %	Cl. septicum %	Cl. Long %	novyi Hall %	Cl. sordellii %	Combined Inocula %
Sodium sulfadiazine	92.0	72.0	4.0	0.0	80.0	88.0
Sodium sulfathiazole	44.0	28.0	0.0	—	32.0	80.0
Sulfanilamide	20.0	0.0	0.0	—	40.0	48.0
Controls	13.2	0.0	4.0	0.0	8.8	6.6
Number of controls	129	31	25	10	34	30

In the therapeutic experiments, inoculation of the culture was made at least three hours before treatment was initiated, and six and twelve hours in certain of the experiments, with a resulting increase in the severity of the infection and in its resistance to the definitive agents. Sulfadiazine, penicillin, and antitoxin, and combinations of these agents, were studied for their curative action in established infections. When sulfadiazine was used as the therapeutic agent against the mixed infection of *Cl. welchii, Cl. septicum, Cl. sordellii,* and *Staph. aureus,* there was an 88 per cent survival in a group of 25 dogs, as compared to a 6.6 per cent survival in a control group of 30 dogs.

From the results given in Table II, the investigators concluded that penicillin and pentavalent antitoxin were both powerful therapeutic agents in experimental gas gangrene, and that antitoxin was the more valuable of the two in the later stages because of its toxin-neutralizing effect. They showed that antitoxin must be used in large amounts, and that the entire dose should be given within a relatively few hours rather than be spaced over one or more days. The possibility of an anaphylactoid reaction was noted, and they suggested that during the period of desensitization sulfadiazine or penicillin, preferably the latter, should be used. The combined use of penicillin and antitoxin proved to be far superior to that of roentgen-ray therapy, and it was concluded that antitoxin should be given in large amounts for control of the toxemia and penicillin for its effect on the local sepsis.

While these prophylactic and therapeutic tests were being made, collaborating investigators studied tissues from the infected control and treated dogs, which indicated that death in these infections was due to toxemia, the chief effect of which was on the heart and liver. The lethal role of liver damage in these animals could not, however, be fully evaluated because

TABLE II

Percentage of Ten-Day Survivals Obtained in Therapeutic Studies
(25 Dogs per Group)

Therapeutic Agents	Treatment Times	Survival Rate %	Remarks
Penicillin	Begun 3 hours after inoculation; continued 72 hours	100.0	———
	Begun 6 hours after inoculation; one dose sodium sulfadiazine 3 hours after inoculation	88.0	———
	Begun 12 hours after inoculation; one dose sodium sulfadiazine 3 hours after inoculation	8.3	Based on 12 dogs
Antitoxin	Begun 3 hours after inoculation; completed in 8 hours	88.0	———
	Begun 12 hours after inoculation; one dose sodium sulfadiazine 3 hours after inoculation	92.0	———
	Begun 12 hours after inoculation; sodium sulfadiazine begun 3 hours after inoculation and continued 72 hours	84.0	———
Penicillin and antitoxin	Begun 12 hours after inoculation (½ usual amount of each); one dose sodium sulfadiazine 3 hours after inoculation	88.0	2 dogs died an anaphylactoid death
Controls		0.0	
Number of controls		99	

studies of the blood chemistry were not done. No definite kidney damage was demonstrated in this disease.

After a series of pilot experiments, the Rochester group, working in collaboration with an investigator at the University of Cincinnati, made an intensive study of the value of immunization with *Cl. welchii* toxoids. These investigations revealed that a blood level of 0.1 unit of alpha antitoxin was the critical titer for the survival of dogs with gas gangrene produced by the injection method. In a group of 23 dogs with titers of less than 0.1 unit, 43 per cent survived, whereas in a group of 51 dogs with titers greater than 0.1 unit, 96 per cent survived. There was a 10 per cent survival rate in the controls used throughout the experiments.

While the investigations at Rochester were being terminated, a new series of experiments was being planned, primarily to study means of preventing effectively and treating successfully a severer form of established gas gangrene produced in animals more susceptible than dogs, and by a method that simulated as closely as possible the conditions on the battlefield that led

to this infection. A method of challenge was needed that could be carefully controlled and could be used to measure quantitatively the effect of the various forms of treatment to be tested.

It has been known since World War I that the organisms causing gas gangrene are ubiquitous. *Cl. welchii,* for example, has been repeatedly isolated from many sources, including the intestinal canal of man and animals, the birth canal in women, dust samples taken from a variety of sources including a warship, soil, sand, and sewage, woolen clothing, and even milk, fish, cheese, and ice. At the University of Cincinnati viable clostridial spores were demonstrated in all samples of street dirt collected from busy street intersections, from twenty-three of twenty-five specimens of wool clothing, and from dust on the floor of various operating rooms, offices, stores, and homes. The ubiquity of these spores explained their high contamination of wounds, but an adequate explanation of the factors determining the development of typical infection was lacking. It became necessary, therefore, to study the effect of various factors on clostridial virulence, and because of its association with 80 to 90 per cent of the cases of gas gangrene, *Cl. welchii* was chosen as the primary test bacterium.

Cultures of this organism grown in a pancreatic digest medium were much more virulent than those grown in deep meat broth, brain broth, or other media. When incubated for only five to six hours, they produced death in much higher dilutions than when incubated for eighteen to twenty-four hours.

A series of experiments was then carried out and repeated, using cultures incubated in pancreatic digest media for four and a half to five and a half hours, in order to measure quantitatively the effect of crushed muscle and dirt in closed wounds on the virulence of *Cl. welchii* (Table III). When pure cultures of the organism were injected into healthy muscle of guinea pigs, it took one thousand times more bacteria consistently to produce a fatal gas gangrene than it did when injections were made into crushed devitalized muscle, and one million times more than when they were made

Table III

Effect of Crushed Muscle and Dirt on Virulence of *Cl. welchii*

Conditions of Experiment	Minimum Lethal Dose of $4\frac{1}{2}$- to $5\frac{1}{2}$-Hour Culture of *Cl. welchii* That Kills All Guinea Pigs Within $4\frac{2}{3}$ Days
Injection of culture only	0.5 cc. of 10^{-2} dilution
Injection of culture in presence of crushed muscle ...	0.5 cc. of 10^{-5} dilution
Injection of culture in presence of crushed muscle and dirt	0.5 cc. of 10^{-8} dilution

into crushed muscle contaminated with a mixture of pulverized sterile dirt and cinders; conversely, it took one millionth as many bacteria to produce fatal experimental gas gangrene consistently in closed wounds in the presence of crushed muscle and dirt as it did in wounds without these substances.

It was thus definitely proved in guinea pigs that the virulence of *Cl. welchii* in a wound was markedly increased by the presence of crushed muscle and dirt, and that the presence of these two factors produced an abnormal physiological state in the wound that favored the development of gas gangrene in the presence of minimal bacterial contamination. This emphasized the practical importance of removing tissues devitalized by injury and of preventing the causation of further devitalization by poor surgery, constricting dressings, or thrombosis of nutrient vessels.

On the basis of these and similar studies, the following method was adopted for the production of a uniformly severe gas gangrene in experimental animals under conditions simulating those of battle. The skin over the lower back and posterior lateral aspects of the thigh of a guinea pig was prepared by shaving, scrubbing with soap-and-water for ten minutes, applying alcohol and ether, and painting with tincture of iodine. After the induction of ether anesthesia, a special sterile drape was applied to the skin and an incision 1 cm. in length was made and carried down to the femur under strict aseptic conditions. The muscles were crushed on each side of the femur five times with a small Kocher clamp and then avulsed and lacerated by twisting the clamp. One cubic centimeter of an autoclaved finely ground mixture of soil and cinders was inserted into the incision, and the edges were closed with interrupted fine black-silk sutures to minimize leakage of inocula and secondary contamination. Finally, 0.5 cc. of one of the various serial dilutions of a culture of *Cl. welchii* representing one or multiples of one minimum lethal dose was injected through the skin into the crushed muscle by means of a tuberculin syringe and a 25-gauge needle.

A series of experiments was then carried out to measure the effectiveness of penicillin in the prevention and treatment of gas gangrene produced by the above method. Penicillin was given intramuscularly every three hours for ninety-six hours after the operation, the first prophylactic dose being administered a few minutes after inoculation of the culture into the wound containing crushed muscle and dirt, and the first therapeutic injection being made six hours after inoculation.

When a standard dose of 2000 units of penicillin per kilogram of body weight was given every twenty-four hours to guinea pigs, 90 per cent of the prophylactically treated group were living twenty-eight hours after bacterial inoculation, whereas only 35 per cent of the therapeutically treated group were still alive (Table IV). However, no animals in either group were alive at the end of four and two-thirds days, indicating that penicillin in standard

Table IV

Prophylactic and Therapeutic Effect of Penicillin on Experimental Gas
Gangrene Produced in Wounds Containing Crushed Muscle and Dirt

Amount of Penicillin Given (units/kg./24 hrs.)	Percentage of Guinea Pigs Surviving When Challenged with 1 or More Measured Minimal Lethal Doses	
	28 hours after operation %	4⅔ days after operation %
Prophylactic treatment:		
2000 units	90	0
8000 units	84	16
Therapeutic treatment:		
2000 units	35	0
8000 units	68	4

doses prolonged the period of survival for a time when given prophylactically, but did not prevent death when given either prophylactically or therapeutically.

When the animals received 8000 units per kilogram per twenty-four hours, which is equivalent to 70,000 units every three hours for a 70-kg. (154-pound) man, the survival rate at the end of twenty-eight hours was 84 and 68 per cent for the prophylactic and therapeutic groups, respectively; at the end of four and two-thirds days it was 16 and 4 per cent, respectively. These results were encouraging, for they indicated that higher doses of penicillin further delayed the progress of this severe form of gas gangrene and increased the interval during which adequate surgery would aid in bringing the infection under control, thereby reducing morbidity and mortality. Such a restriction of the invasiveness of the infection would be desirable during combat, when there is frequently a time interval of several hours to days between the time of injury and the time the wounded soldier reaches a unit where definitive surgery may be performed. The above experiments were also of importance since they suggested that much higher doses of penicillin might give further protection, but there was no evidence that chemotherapy with penicillin would replace adequate surgery in the control of gas gangrene.

Studies were begun on the prophylactic value of toxoid immunization [1] against this severe form of gas-gangrene challenge in guinea pigs. Time did not permit the completion of these experiments, but early results indicated that immunization by two and three average doses of toxoid protected only 20 to 40 per cent of animals challenged with one minimal lethal dose injected into a wound containing dirt and crushed muscle.

[1] For detailed discussion concerning the development and testing of toxoid, see Chapter II.

Earlier diagnosis has been facilitated by a rapid method developed at the University of Cincinnati for the identification of *Cl. welchii* in a wound within five to eight hours. There are, however, no tests available that indicate beforehand the wounds that will or will not develop gas gangrene. The importance of some of the factors in the production of this infection has been well defined, but the etiologic significance of others, both known and unknown, remains obscure.

There is strong evidence that the gas gangrene produced in the presence of devitalized muscle and dirt is a form of infection radically different from that produced by the injection of bacteria into healthy muscle. Not only is it severer, but it is more refractive to prophylaxis and therapeusis.

Methods must be developed to distinguish the specific toxemia due to clostridial exotoxins from the nonspecific toxemia caused by toxic products arising from the septic decomposition of devitalized tissue, in order to understand better the pathological physiology of the body and its tissues in gas gangrene. Significant work on this aspect of gas gangrene was performed during the war by a group of British investigators.

Although considerable advancement has been made in the knowledge of the nature of gas gangrene and in its prevention and control, many problems still remain unsolved. A method of immunization by the injection of toxoid for the prevention of gas gangrene in wounded persons must be developed. The results of preliminary experiments in animals and volunteers are encouraging, and they indicate the probability of perfecting such a method. The exact value of univalent and polyvalent antitoxins in the prevention and therapy of gas gangrene has not been definitely shown. Further studies are indicated to explore the value, limitations, and adequate dosage of the new antibiotic agents in the treatment of gas gangrene. It is essential that problems such as these be solved by further investigations if the still prevailing unsatisfactory morbidity and mortality rates for gas gangrene are to be reduced.

THE BURN PROBLEM

OLIVER COPE

THE ATTACK on Pearl Harbor caught the surgeon as well as the armed forces off guard; he was baffled when confronted by so many and so severely burned casualties. His consternation, coupled with the reported incidence of burns among the casualties of the bombed civil population in Great Britain and of the soldiers in desert warfare, made the management of burns an immediate concern of the Committee on Medical Research. A conference was called at the headquarters of the National Research Council on January 7, 1942, at which a standard system of burn therapy, based on the knowledge available at the time, was outlined for immediate guidance of the armed services, and investigation into improved methods was stimulated. In the four years after that conference, extended investigations concerning the treatment of the surface wound, the nature of the disordered physiology, and the control of infection in burns were carried out. They entered diversified channels and covered a wide range of method and point of view, both in the experimental laboratory and in the clinic. Owing to an emotional desire to aid the war effort, some channels, like fashions, were followed blindly; others were left too long unexplored. The accomplishments, however, are many and are attributable to a vigilant concern with the more fundamental aspects of each problem.

SURFACE TREATMENT

A fanatical concentration on the surface wound in burns has been supplanted by a healthier attitude, which recognizes that the amount of attention to be accorded it may be minimal and should at all times be proportional to what the patient as a whole will gain from it. This sense of proportion in the care of the wound has been manifested by a trend away from complexity of treatment and injurious substances toward expeditious closure of the wound and the recognition of the limitations of pressure therapy.

From the reports of the treatment of burn casualties at Pearl Harbor, it was obvious that the method used in the treatment of surface wounds was too complex. Too much of the time and attention of the available medical personnel was devoted to care of the wound, to the exclusion of treatment of

the internal economy. If without prejudicing good care the surgical ma-
neuvers could be minimized, the modest numbers of personnel available in
warfare could care adequately for a large number of casualties. The most
time-consuming maneuvers were those attending débridement, cleansing,
and repeated spraying of coagulants, and it was therefore in order to see
whether any of these could be dispensed with.

Were débridement and cleansing necessary for the care of the burn
wound? This problem was attacked by observing burn wounds in patients
in which the only local treatment was the application of a protective gauze
dressing. At intervals, bacteriologic and chemical analyses were made of the
wound fluid. In burns of incomplete thickness, of which blebs are the usual
accompaniment, it was found that so long as the roof of a bleb remained
intact, little or no infection developed, and that removal of the roofs of blebs,
as generally practiced in débridement and cleansing, was therefore not only
unnecessary but contraindicated. It was also found that no amount of wash-
ing and scrubbing of the wound left after rupture of the blebs removed all
the bacteria, and that the substances commonly used as detergents on the
wound caused cellular damage in experimental animals.

Chemical analysis of the blood stream and bleb fluid showed that a chemo-
therapeutic agent, a sulfonamide, when given systemically permeated into
the burn wound. This permeation fortified the concept of omitting débride-
ment and cleansing. If blebs ruptured in spite of a protective dressing, the
chemotherapeutic agent was available to combat any organisms admitted
to the wound.

Could coagulants be dispensed with? The treatment of a burn wound by
the application of a coagulant to the surface was introduced by Davidson
in 1926. He used tannic acid, which he sprayed on repeatedly during the
course of twenty-four hours or until a firm, leathery eschar had formed; this
eschar was the sole protection of the wound. Davidson believed that the
tannic acid fixed in situ any toxins that might be elaborated in the wound,
and also that it did not damage the viable cells at the base of the wound.

The use of tannic acid was re-evaluated not only because it added to the
complexity of the treatment of the surface wound of a burn, but also because
it seemed possible that the coagulation it produced increased the depth of
skin destruction. Davidson's assumption that tannic acid had a preferential
respect for viable cells seemed unreasonable on chemical grounds and, fur-
thermore, its absorption had been suspected of causing liver damage.

The toxicity of tannic acid, both locally in the wound and on the internal
organs when absorbed, was tested. When applied on wound sites in human
beings from which skin for grafting had been removed, it delayed healing.
In experimental wounds it caused local necrosis, and its absorption resulted
in liver necrosis. It was thus demonstrated that tannic acid did kill viable
cells and would therefore, when applied to a burn wound, increase the

damage done by the burn. It was therefore considered wise to dispense with tannic acid and to find some other substance to apply to burn wounds.

Numerous substances were tested, both on human beings and on animals, in the effort to discover a suitable dressing for the burn wound to supersede tannic acid. No substance was found that would promote wound healing; from the substances tested that did not injure viable tissue, mineral-oil petrolatum was chosen as the most practical. A simple treatment for the burn wound that relegated its care to its proper place by avoiding unnecessary complexities was therefore available. It consisted of a protective gauze petrolatum dressing, to be applied under aseptic precautions but without cleansing or débridement and to be accompanied by systematic chemotherapy. The surgical maneuvers of such a dressing are minimal and can be applied by the relatively untrained, leaving the trained personnel to concentrate their attention on the administration of chemotherapy and the treatment of shock and other exigencies.

Before the war, the medical profession was overwhelmingly prejudiced in favor of the use of tannic acid and its attendant ritual of cleansing and débridement. Of all the doctors at the Burn Conference in January 1942, only three raised a voice against tannic acid, and one of these was in favor of another coagulant. In the first year of the war several of the co-operating burn research groups explored methods other than the tannic acid, including protective-dressing methods with pressure dressings. It was not until the Cocoanut Grove fire occurred in Boston, however, that a sufficient number of burn patients were treated by the simplified method without débridement and cleansing to warrant its general acceptance.

A unique opportunity was afforded by this disaster to compare this method with others. All the patients entering the Massachusetts General Hospital were treated by the protective-dressing, no débridement and cleansing method; those at the other hospitals were treated by cleansing, débridement, and the administration of tannic acid or other coagulant dyes. A survey of the results of the treatment of the two groups revealed that the administration of plasma and chemotherapy could be more prompt when the surface treatment was simplified, and suggested that the healing of the wound was satisfactory and that wound infection and renal damage were minimal. The economy in the use of the trained personnel was clear-cut. The method was recommended by the National Research Council for use by the armed services.

The second phase of investigation concerning the surface wound was directed toward the expeditious closure of the deep or full-thickness burn wound. The simplified method permitted prompt healing with minimal infection of wounds with residual viable skin, but it did nothing, beyond combating invasive infection by systemically administered chemotherapy, to aid in the healing of wounds in which all the skin elements had been de-

stroyed by heat. The dead tissue of these wounds, in the simplified method as well as in the tannic acid method, was allowed to slough spontaneously or with the aid of wet dressings; grafts were applied whenever there was vigorous proliferation of fibrous tissue or formation of healthy granulation tissue. Not less than four weeks was usually spent in waiting to graft, and the time was often much longer when infection flourished. If the full-thickness wounds were extensive, the patient had to be hospitalized for many months before all the wounds were closed. Prolonged infection in the slough and open wounds led to debility and malnutrition, to scarring and limitation of joint motion, and to psychological discouragement on the part of the patient.

If the necrotic tissue of the full-thickness burn wound could be removed to permit prompt grafting, not only would hospitalization be shortened and casualties be returned to active duty with speed, but also the entire period of debilitating infection might be circumvented. With this in mind, two projects were inaugurated. In one the attack was primarily chemical; in the other it was surgical.

In the chemical project, deep burn wounds were produced in dogs and various acids were assayed for their ability to dissolve the slough. Many acids were found that would accomplish the dissolution, but pyruvic acid, a product of the physiological breakdown of glucose, alone left a base suitable for grafting. Application of this acid in a starch paste to the full-thickness burn wound for only three to six days was sufficient to remove the slough and permit grafting.

In the surgical project, the area of full-thickness destruction in patients was excised and grafts were laid on the freshly exposed viable tissue at the base of the wound. It was found that such grafts took without fail if the excision was carried out within forty-eight hours after injury, before infection had had time to develop. Since several days might elapse under conditions of warfare between the time of injury and the arrival of a casualty in a hospital with facilities for surgical procedures, the operations on civilian patients were also delayed for days arbitrarily. Penicillin was given to these patients during the period of delay, as it could be to the casualties in the armed services. It was found that although penicillin was able to hold invasive infection in abeyance, it was not able to prevent bacteria from growing in the slough and infection from developing in the contiguous portions of the wound. Therefore the longer the excision of the slough was delayed, the less probable was the success of the grafting.

There are thus two methods available to expedite the convalescence of casualties with full-thickness burns. By both, the anticipated results have been achieved; the periods of disability and hospitalization have been drastically reduced; less scarring, deformity, and debility have been encountered; and the patients maintain a normal psychological outlook throughout

convalescence. On the other hand, each method has its limitations. The pyruvic acid dressings are cumbersome to apply under conditions of warfare and require careful supervision. Surgical excision requires an understanding of what is full-thickness destruction, because otherwise the wound will be enlarged unnecessarily. Such knowledge is gained only with experience, since no means of designating the depth of a burn has been developed that is better than the unaided eye. Also, operation is contraindicated in an extensively burned patient unless the surgeon is confident that physiological balance can be maintained.

Both these methods were developed in the last two years of the war, and neither was accepted as part of the routine advised by the National Research Council for use by the armed services. Nevertheless, their development constitutes a distinct advance for the care of civilian burn casualties.

At the beginning of World War II, much was hoped for from the use of pressure dressings in burns of the head and extremities. Having been employed as an adjunct to plastic operations, they had prevented postoperative hemorrhage and edema and had apparently minimized scar formation. It was believed that the coagulation of the protein of edema fluid in the operative wound led to increased fibrosis, and that this would also form in a burn wound if the edema fluid were allowed to collect. It was postulated that prevention of the collection of edema in a burn wound by external pressure would prevent, or at least reduce, the amount of plasma fluid escaping from the circulating blood. Such a sparing effect on plasma loss should reduce the likelihood of burn shock.

Experimental and clinical observations failed to confirm unequivocally either point. Burn wounds allowed to swell unimpeded healed with no more scarring than did those in which swelling was prevented by an elastic or plaster bandage. Although edema was successfully prevented from forming in the wound by the pressure, it did collect proximal to the dressing, on the upper portion of the extremity, around the neck, and on the trunk where pressure could not be applied. Such collections suggested that the edema fluid was merely displaced and that plasma loss had not been prevented. The critical experiment to prove that a pressure dressing does exert a partial sparing effect on plasma loss is still to be devised.

DISORDERED PHYSIOLOGY

The investigations into the nature of the disordered physiology of burns during World War II have been primarily concerned with shock, organic dysfunction, and malnutrition. There is a noticeable trend in point of view from one limited to the extracellular space to one that includes the cell itself.

In regard to shock in World War I, research disclosed the importance of maintaining an adequate circulating blood volume. In the period between

the two wars it was realized that the salient defect in burn shock was a decrease of plasma volume, and that the fluid lost from the circulating plasma disappeared into the dilated interstitial space of the wound. In World War II the nature and extent of redistribution of fluid within the body were measured and a better fluid for therapy was sought.

In order to anticipate the volume of fluid needed for therapy, it was necessary to know just how great the loss of plasma volume was and how much the interstitial space increased in size. Prior to the onset of hostilities, the needs of a burned patient had been judged by repeated determination of the hematocrit, red-cell count, or hemoglobin concentration. The surface-area formula evolved at the Burn Conference one month after the attack on Pearl Harbor was based on theory. It was therefore important to compare this formula with others in practice.

The redistribution of fluid following burns was studied in four projects. It was soon found that there was so much variation from patient to patient that no one formula was altogether satisfactory, and that although the surface-area formula was the best it was still only an approximation. It was only in the last year of the war, when adequate data had been assembled, that it was realized that there is a limit to the expansion of the interstitial space, and that the relation between this enlargement and the extent of the burn is not a linear one.

The importance of being able to anticipate the volume of therapy needed by a burn patient has been shown by the pulmonary complications occurring in patients who have received overenthusiastic therapy or have suffered pulmonary damage from noxious gases from fires in enclosed places. Death from pulmonary edema has been encountered in such patients and a warning sounded.

The search for a fluid better than plasma and saline solution for the replacement therapy of burn shock has run the gamut of colloid and electrolyte solutions, but a wholly satisfactory one has not been discovered. Most of the work has centered on a comparison of the effectiveness of albumin concentrates, various solutions of the sodium ion, gelatin, and whole blood with that of plasma and isotonic sodium chloride solutions. The difficulty inherent in the problem does not lie in maintaining an adequate circulation during the phase of development of wound edema, for many colloids will accomplish this. The difficulty lies rather in getting rid of the fluid given as therapy once resorption of wound edema has begun. The interstitial space of an extensively burned patient may have been enlarged by as much as 10 liters of fluid. The presence of an excess of colloid prevents prompt excretion of the fluid by the kidney during the phase of resorption. The plasma volume therefore increases above normal and may rise to the extent of producing fatal pulmonary edema. What is needed is a colloid that remains stable for forty-eight hours and then rapidly disintegrates, or

one that does not pass out through the mutilated capillary walls of the burn wound.

The colloid of the concentrated albumin solution passes the capillary membrane and is destroyed at the same rate as plasma albumin, from which it is derived. Furthermore, concentrated albumin has a dehydrating effect on unburned tissues. Although gelatin was shown to be a highly useful substitute for plasma in the management of burn shock, interest in it (as well as in plasma) waned when it was demonstrated that the proper therapy for many burn patients includes the early replacement of red cells lost from the circulating red-cell mass. Thus, whole blood began to be added to the treatment of burn shock in the latter period of the war years. Whole blood for burn shock when transfused in excess of the red cells destroyed increases the circulating red-cell mass, since the plasma, not red cells, is lost by seepage into the wound. With the rise in proportion of red cells, the viscosity of the blood increases; such a rise from transfusion of red cells has been shown in dogs to be associated with a drop in cardiac output.

In spite of the promising initial clinical trials with whole blood, it remains to be determined how far the human being can tolerate an abrupt rise in hematocrit. This may not prove to be a problem in the severely burned patients because in these the relatively rapid destruction of red cells during the immediate post-burn period tends to prevent the occurrence of hematocrit values high enough to influence viscosity significantly. An advantage of whole-blood therapy is that it tends to prevent the early development of anemia in the severely burned patient.

A deal of emotion, energy, and time has been spent in re-exploring the usefulness of the sodium ion in shock. Discarded in World War I as inadequate, it has again been brought to the fore by experiments on mice. Its reintroduction in the clinic in the treatment of burn shock has been championed by more than one group. To scrutinize the need of the burned patient for sodium, an elaborate clinical project was established. A radioactive isotope of sodium was injected into burned patients at varying intervals following injury. In addition to measuring the distribution of sodium in the extracellular fluid, it was also measured within cells of excised burned and unburned skin. Although sodium was found to replace the potassium of the damaged cells, the need for sodium on this basis was slight, even in a patient with a burn of more than 50 per cent of the body surface, because of the small intracellular phase of skin. The amount of sodium required therefore remained largely that needed to fill the enlarged interstitial space. The discrepancy between the experiments on mice and the observations in human beings is to be explained by the damage to muscle in the burned mice.

The burn trauma itself and the impaired circulation of burn shock may derange endocrine balance and disturb the cell function of organs. Burns, as well as other forms of trauma, may initiate the so-called "alarm reaction,"

and the chain of events leading through the anterior pituitary and adreno-cortical glands to the lymphoid apparatus is of much interest to the surgeon caring for burns. The reaction, described first in experimental animals, has been encountered in full form in the burned human being, even to the excretion in the urine of excessive amounts of adrenocortical-like hormones. The meaning of the reaction is not yet clear, but it is certainly part of the adaptation of the healthy person to an unexpected insult, since a patient who was ill prior to being burned fails to exhibit the reaction in the manner of the previously healthy person. Investigations into the nature of this alarm reaction, cut short by the end of the war, should be continued until its purpose in the body economy is clear. Leads for methods of expediting wound healing and combating infection are certain to be forthcoming.

The damage to the kidney and liver by the impaired circulation of shock has been the subject of extensive investigation. The importance to fluid and electrolyte control and to intermediary metabolism of preventing circulatory collapse has been clearly demonstrated, both in the experimental animal and in the clinic.

MALNUTRITION

Destruction of tissue, loss of body fluids and proteins from the wound surfaces, infection in the wounds, and disturbance of the gastrointestinal tract, if not corrected, lead rapidly to malnutrition. A stubborn anemia is encountered which stems from a continued disappearance of red cells within the body and cessation of bone marrow activity. Plasma proteins likewise decrease in concentration. The need for certain vitamins is increased.

What part the alarm reaction plays in malnutrition is not understood. An inherent result of the reaction appears to be an increased excretion of nitrogen in the urine. The source of this nitrogen has not been determined. That it may not come from cells is suggested by the absence of a parallel loss of potassium and phosphorus. The disturbance in nitrogen balance, therefore, may involve only the proteins, such as those of the plasma, which are not an integral part of cell structure. Generalized wasting, however, which includes muscle tissue and is accompanied by loss of strength, suggests cell involvement.

Investigation in the field of nutrition in relation to burns has been cut short by the close of the war. It must be pursued in peacetime, for it has finally focused attention, heretofore concerned only with the extracellular fluid, on the functioning and requirements of the cell.

⌐ INFECTION ⌐

Infection of the burn wound is still a dreaded complication. Although progress in its control has been accomplished by the introduction of the sulfonamides and penicillin and by early eradication of slough and grafting of full-thickness wounds, infection remains a stubborn accompaniment of the deep wounds of extensively burned patients.

At the beginning of the war the sulfonamides were believed to be the wonder drug of the age. The initial report from Pearl Harbor implied that they had revolutionized military surgery. Such faith, useful for purposes of morale in the fighting forces, seemed unjustified in the light of previous laboratory and clinical experience. Fortunately, six months before Pearl Harbor a collaborative enterprise involving ten hospitals had been started to study the effectiveness of the sulfonamides in the control of infection in traumatic and burn wounds. With pooled evidence increasing the statistical significance, it was early apparent that sulfonamides locally applied to open wounds such as burns did not decrease the incidence of infection. Initially this finding was given small credence, but it has been fully appreciated with time and more evidence. Invasive cellulitis and lymphangitis due to the streptococcus, a frequently encountered type of infection, fell a prey to these drugs, but infections due to other organisms were less if at all controlled. Despite this limitation, the sulfonamides undoubtedly represent a step in advance in the therapy of burns.

The introduction of penicillin has been an even more distinct step forward, because it has controlled, if not eliminated, the most destructive infection of burn wounds, that wrought by the staphylococcus. Penicillin has made it possible to eradicate the invasive infection of this organism, even though it has not been possible to eliminate it from the burn wound. The effectiveness of this drug, however, has been found to be limited by the presence of organisms that are not susceptible to it or that inhibit its action, by the presence of dead tissue, and by the development of resistance by organisms ordinarily susceptible. Because burn wounds are on the outer surface of the body and therefore exposed, they are subject to contamination from many sources and a variety of organisms. The longer a wound remains unhealed, the greater is the chance of there being organisms in it that are either un-amenable or unfriendly to penicillin. Since penicillin does not penetrate sloughing dead tissue to kill the organisms thriving inside, infection has been reduced in deep burn wounds by eliminating the slough. In extensively as well as deeply burned patients, however, it may not be possible to obtain enough skin for grafts, and large wound areas will remain in which infection smoulders in spite of the penicillin. In such open wounds the staphylo-coccus gradually becomes resistant to the drug, and although invasive infec-

tion does not result if adequate penicillin is administered, the infectious process may flourish to the extent of precluding successful grafting. The conquering of the infection will depend on the development of immunity by the patient.

Recounting the limitations of the use of penicillin in burn wounds should in no wise detract from the merit of this drug. An attempt has been made to show that in the course of investigations of its use in burns it has been realized that penicillin cannot eliminate infection. The drug is only an adjuvant to diligence in reducing bacterial contamination and to timely surgical measures.

THE REPAIR OF PERIPHERAL NERVE LESIONS

LEWIS J. POLLOCK

ONE OF THE most frequent injuries sustained in war is that of the peripheral nerves. It is also one of the most disabling. The reason for this is that the functions of nerves are varied and complex; they carry impulses of sensation from the skin and other tissues, of motion to muscles, of control of circulation of blood, and of secretion. They literally supply life to all tissues. When a nerve of an extremity is severely injured or torn apart, the extremity becomes paralyzed and anesthetic. It wastes away, circulation becomes faulty, secretions stop, and ulcerations of the tissues may occur.

Another reason for the serious disability caused by nerve injury is that, unlike skin, muscle, or bone, nerves do not heal by the joining of their torn edges. When skin is torn and the edges are placed in apposition, they rapidly grow together and function is immediately resumed; not so with the nerve. When it is torn apart, all the fibers distal to the point of injury rapidly disintegrate and disappear. In this case not only must the nerve ends grow together in some form of scar, but the nerve fibers, which arise from cells in the spinal cord, must grow through the scar and at a certain rate into the distal segment until they reach the muscles, sensory endings, and so forth before function can be resumed. Still another reason is that before the fibers can grow into the distal segment, the nerve ends must be brought together and skillfully joined by surgical treatment. If this were not done, a few fibers would find their way into the distal segment, but not enough would do so to restore adequate motion or sensation. At times portions of nerves are shot away and their ends cannot be approximated because of the large defect and because nerves cannot be stretched. Methods for bridging such a gap must therefore be devised.

It is unfortunate that muscles become just as paralyzed, and skin just as insensitive, when the nerve is injured by being compressed or concussed as when it is torn apart, since in the former case it often heals without operation. Other methods for ascertaining what has happened to the nerve must be developed.

A nerve fiber grows about 5 mm. a day; in other words, it takes five days to grow an inch. Before it is repaired and during all the time it is growing after repair, the tissues continue to waste and harden. The sooner and the

better it is repaired, the less will be the residual disability. It is necessary, therefore, to learn when to operate, what cases to operate on, and how best to join the severed ends, and finally to find ways of encouraging growth of nerve and discouraging wasting of muscle, hardening of tissue, and so forth.

Differing from injuries in civil practice, injuries of the nerves received in war are associated with widespread destruction of adjacent tissue, muscle, blood vessels, and bones, and there is always exposure to infection. The question of whether to join nerves as soon as possible after injury, despite infection, becomes important. Also important is the prevention of infection.

Many lessons were learned during World War I, both from experimental work and from military practice, but much remained to be discovered and some errors needed to be corrected. The failure to follow up the results of operative treatment of wounded men left us at a disadvantage in formulating ideas of surgical treatment. In World War II the establishment of a special recording center, known as the Peripheral Nerve Registry, in the Office of the Surgeon General of the Army permitted follow-up studies, which were often of great value.

The problems posed to the Subcommittee on Neurosurgery of the National Research Council at the beginning concerned the causes of nerve injury; the prevention and treatment of infection; methods for joining severed nerves, including those for bridging gaps; the use of various types of sutures and glue; the effect of delay in suturing; studies of the effects of nerve injury, paralysis, anesthesia, atrophy, contractures, ulcerations, and sweating; studies of the microscopic appearance of nerves and muscles to learn the effects of infection, of various types of treatment, and of operations; development of methods of treatment of wasted muscles and of contractures; studies of the effect of treatment by drugs, vitamins, and so forth; and improvement of old methods and developments of new ones for ascertaining when a nerve was torn apart or was growing satisfactorily. Concentration and intensive research led to the solution of some of these problems. Some remain to be solved, but the impetus for their solution will carry the work over from the Office of Scientific Research and Development into different laboratories.

COMPRESSION AND SEVERANCE OF NERVES

In many cases of paralysis, the cause is not a laceration of a nerve but compression, as by a callus or a tourniquet or concussion, when a missile passes near a nerve but does not sever it. Frequently only blood vessels are injured.

Researches carried out under OSRD(CMR) contracts indicate that varying degrees of compression, as by clips placed on nerves for variable periods of time, produce a characteristic form of paralysis, which, dependent on the

force of its compression and the length of time it is exerted, ranges from a delayed temporary motor paralysis with rapid recovery to early paralysis and gross defect in sensation, also recoverable. The effect is considered to be due to obstruction of circulation to the nerve. The reason for rapid recovery was found in the fact that there was no disappearance of nerve fibers below the level of the compression, but only a swelling and disappearance of the myelin sheath about the fibers. Below the level of injury the nerve could be stimulated electrically, and impulses to muscles would thereupon produce motion. It was also found that despite paralysis no wasting of muscles occurred because the nerve was connected with its cell of origin in the spinal cord. By tying off the artery that supplied blood to the nerve, results similar to those of compression could be produced. Although high-altitude freezing is known to produce motor and sensory loss, direct cooling of a nerve resulted only in loss of motion, not in loss of sensation.

Of great significance was a report showing that in contrast to the cutting of a nerve by a sharp knife, its severance by a bullet produced widespread destruction of the nerve above and below the point of injury, in some cases for almost an inch. Such destruction leads to faulty regeneration unless before suture these necrotic parts of nerves are resected.

RESULTS OF NERVE INJURIES

Reports on experimentally produced injury to nerves revealed the same losses of function as are seen in injuries in man. When muscles are paralyzed, other uninjured ones often imitate the movements that usually occur when the paralyzed muscles contract ("trick movements"). Likewise after sensation is lost, adjacent nerves at the borders of sensory loss supply a single area for a small distance; furthermore, as confirmed by microscopic studies, sensory nerves from adjacent uninjured trunks grow into anesthetic areas and recovery of sensations evoked by deep pinprick may occur, although the injured nerve does not regenerate ("sensory overlap").

When a nerve supplying a muscle is severely injured, the muscle wastes. This atrophy had been known to be associated with fibrillation, which is a condition in which by discrete, local, minute contractions the muscle seems to be in a continuous shimmering activity. As will be seen, this activity can be demonstrated by changes in electrical potential produced with each contraction and recorded on a cathode-ray oscillograph. From the fact that atrophy began before fibrillation, it was proved that fibrillation is not the cause of it. Progressing as an exponential function, atrophy proceeds at various rates in different species, most rapidly in animals of short lives, as rats, and less rapidly in others, as cats and monkeys. Prostigmine increases fibrillation but not atrophy.

It was shown that although ulceration occurred in anesthetic parts of the

extremity, ulcers might not have healed when sensation returned and might even develop subsequently. The amount of atrophy was unrelated to complicating ulcers, contractures, motor recovery, or signs elicited by electrical examination. Atrophy of bone did not occur as the result of immobilization or injury to the nerves. It did occur when there was an ulceration of overlying tissues, which often recovered before sensation returned. The specific gravity of muscles diminished after nerve injury and returned to normal with recovery of the nerve.

Immobilization is not the sole cause of contractures; when a nerve is injured and its muscles are kept in a shortened position, contractures occur. They also occur if the extremity is not held in any prolonged fixed position. As recovery of the nerve occurs and voluntary motion returns, the contractures disappear.

When sweating ceases as the result of a nerve injury, the electrical resistance of the skin becomes greatly increased. By devising a simple ohmmeter it became possible to outline the borders of loss of sweating. In general, this area corresponded to that of sensory loss. Adjacent uninjured nerves supplied function to the borders of these areas and also grew into them as time progressed. Actual recovery of sweating occurred after recovery of sensation and motion. By the use of this method in military hospitals significant information was obtained that assisted in the diagnosis of injured nerves.

ELECTRODIAGNOSIS

Clinical methods for studying voluntary motion and sensation do not differentiate between injuries by compression or concussion and those associated with severance of a nerve. In the former case recovery may occur without operative treatment; in the latter, surgery is necessary. In World War I, it was hoped that a study of the response of muscle to electrical stimuli would serve to differentiate the two states; this hope was not fulfilled.

A number of such investigations were made. They consisted of studies of direct stimulation of nerve, both experimentally and at the operating table, and of direct muscle stimulation; of recording of action potentials of nerve and muscle; and of stimulation of muscle through the skin by currents of variable wave forms, duration, frequency, and intervals.

OBSERVATIONS ON STIMULATION

Many important observations were made on conduction and conduction velocity, which will when studied further clarify some questions. The response of the nerve to stimulation at the operating table served to prove its recovery when clinical methods had failed. The amount of current necessary to produce a contraction of muscle stimulated through the skin, when plot-

ted against such factors as duration, frequency, and interval, produced curves that were characteristic for degeneration, denervation, and regeneration.

Modern electronic methods permitted the collection of accurate data leading to means of differentiating recovering from irrecoverable injuries. A number of devices for stimulation and measurement were invented.

When an injured nerve failed to grow into the muscle that it formerly supplied, certain characteristics were noted. The muscle responded to very small amounts of current of long duration (low rheobase), but extremely large amounts of current were required to stimulate it when the duration was very short. Thus, when one determined the duration of a current necessary to stimulate at twice the amount for a very long stimulus, the time required was much longer than in normal muscle (long chronaxie). In the case of the normal muscle, the amount of current necessary to produce a sustained contraction during the time current is flowing through the muscle is four or five times that necessary to produce a twitch on closing the circuit. In contrast to this, the denervated muscle responds by a sustained contraction at the same value of current as is necessary to produce any movements. This characteristic, measured as a "galvanic tetanus ratio," led to a diagnostic procedure whereby irrevocably damaged nerves were recognized on the one hand and progressive improvement followed operation on other nerves on the other hand. Recovery could be predicted as long as five months before return of voluntary motion. This method was employed in large numbers of cases at the Percy Jones General Hospital in Battle Creek, Michigan. Many stimulators were built there and distributed among Army hospitals, after medical officers had been trained in their use. The method was also used in some naval installations.

In addition to the studies described, others, such as those on strength frequency curves, strength duration curves, strength interval curves, and progressive currents, were also made.

Electromyography

As stated above, a short time after a nerve is severely injured the muscles it formerly supplied enter into a state of continuous but minimal activity (fibrillation). These minute repeated contractions produce a difference in electrical potentials, which, when electrodes are placed in the muscle and the current is led into an amplifier and then into a cathode-ray oscilloscope, may be recorded as characteristic wave forms. The current may likewise be led into a loudspeaker and produce characteristic sounds. This procedure, known as electromyography, led to important conclusions. When the muscle again received a nerve supply, although attempts to contract it produced no visible movement, certain bursts of action potentials occurred in a form that constituted the surest sign of recovery.

Fortunately, the Canadian Army permitted Captain Herbert Jasper to bring his electromyograph to the Percy Jones General Hospital, in Battle Creek, Michigan, there to study a large number of cases being investigated by other electrodiagnostic methods. This led to the building of electromyographs by others and to continuation of studies at the Percy Jones Hospital and certain naval installations.

TREATMENT

SURGICAL PROCEDURES

It is the consensus of opinion that the earlier an injury to a nerve is repaired, the better is the opportunity for good recovery.[1] Therefore, the question whether an infected field contraindicated surgical repair was one of the most serious ones. It was found that unless animals died as the result of an extremely severe infection, nerve regeneration and functional recovery were not influenced by contamination of the wound. However, since infection could lead to other deleterious effects, and even death, it was necessary to study the effect of treatment by such drugs as the sulfonamides.

Microscopic studies showed some effects of these drugs, but their local use appeared to cause little interference with regeneration of a well-sutured nerve. The introduction of sulfonamides into a wound permits earlier repair of an injured nerve. If sulfathiazole jelly is placed in a wound at the time of its inception, débridement and repair of a nerve can be delayed for as long as twenty-four to thirty-six hours without development of infection.

In many cases treated before and during World War I good recovery did not follow surgical treatment. The question of the best method for joining nerve ends was restudied. In addition to types of suture material (silk, tantalum wire, or various forms of plasma acting as a glue), studies were also made on the use of various tubes (arterial, collagen, or tantalum), cables of fibers of synthetic resins or glass wool, and so forth. Since it is impossible to prevent some regeneration of a severed nerve whatever obstacles one interposes, it was to be expected that varying degrees of success would follow any of these methods. Continued reports from military hospitals, however, indicate that despite good results in experimental animals, tantalum, especially as a cuff, probably should not be used.

Microscopic studies indicated that by the use of plasma the fibers growing through the point of injury assumed a more orderly form. Whether this actually contributes to the degree of successful return of function remains to be seen. Nevertheless, successful results were observed, both in experimental animals and in wounded men. For purposes of nerve suture, autologous un-

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[1] There are, however, some opinions to the contrary, notably in England.

modified plasma is preferable. Special and ingenious instruments were devised to improve apposition of the ends of severed nerves, for trimming nerve ends, and for obtaining hemostasis.

Quite frequently it is found that in the wounds sustained in war a large section of a nerve has been destroyed and that the ends are so far separated that methods must be devised to permit their joining. In World War I, experimental work gave ample proof that many types of so-called grafts, obtained from other nerves of the animal studied (autogenous grafts) and from other animals of the same species (homogenous grafts), as well as various tubes, Cargile membrane, blood vessels, and stored fixed nerves, might lead to variable degrees of success in experimental animals. In contrast to this, the use of grafts in the repair of human nerves led to discouraging results. This brought a conviction that they were useless, a belief that influenced surgeons during World War II to use grafts very sparingly. Nevertheless, the problem of repair by grafts was attacked from the beginning by investigators under OSRD(CMR) contracts.

From the experimental standpoint the successful results previously obtained were repeated. Although recovery of voluntary motion, sensation, and muscle bulk was slower than with primary suture, it occurred after autogenous and homogenous grafts in most cases. In a certain number, there was necrosis of part of the graft. Some investigators advised cable grafts, the strands at their ends being joined with plasma. It was shown that regenerating myelinated fibers may be limited by the smallness of Schwann tubes into which they may grow. Therefore, when nerve grafts are used, the diameters of the nerve fibers of the graft should not be less than those of the host nerve.

Further work is indicated to determine the possibility of impeding growth at the distal suture line, and further evidence is required regarding the value of grafts in human cases.

Since in practice it has been found that a long nerve can be dissected from almost all of its bed with impunity, and since by this method, combined with transplantation of the nerve into a bed with a shorter distance, ends of nerves may be joined despite long defects, there will be less indication for the use of grafts. However, the impatience that leads to discouragement and to reoperation at intervals too short to permit recovery should be warned against.

In many cases, nerve repairs are delayed because of infection, lack of proper surgical facilities, and injury to other parts. During World War I it was found that the longer was the delay before repair of a nerve, the less successful was regeneration. Histologic studies by some investigators showed that delays up to ninety days had no noticeable effect on regeneration in the distal segment with regard to diameter or quantity of regenerating fibers. Others found that a delay of sixty days resulted in greater atrophy, a longer period of recovery of muscle bulk, delayed or incomplete motor recovery,

and delay in sensory recovery. In only a few cases did sensation completely return. There were a larger number of contractures, they were severer, and their recovery was delayed.

OTHER THERAPY

One of the most pressing questions related to the prevention of atrophy and facilitation of recovery of muscle bulk. Prior to World War I, opinions regarding the efficacy of electrotherapy differed widely among physiologists and clinicians. After the beginning of World War II, British investigators reported that in the rabbit atrophy was retarded and disappeared more quickly after suture when the muscle was treated by electrotherapy. Investigators in this country later reported that in the cat no statistically significant difference was found in the amount of atrophy that occurred after section of a nerve or in the rate of its disappearance as between animals treated by electrotherapy and those not so treated, provided that the animals were allowed to walk about unimpeded by casts or splints. On the other hand, if the extremity was immobilized, atrophy was greater and recovery was retarded when electrotherapy was not used.

Other investigations on the rat and the rabbit demonstrated that electrotherapy was efficacious. This led to the conclusion that the difference in results was caused by difference in species. Further work in this field is indicated.

Massage and passive movement resulted in some facilitation of return of motion and retarded the development of contractures. It also aided in hastening their disappearance.

In spite of severe vitamin B_1 deficiency produced in cats, the oscillographic analysis showed regeneration to be excellent. The addition of this vitamin to the diets of other animals with adequate diet had no effect on regeneration. Treatment by Prostigmine Bromide had no effect on regeneration.

MICROSCOPIC EVIDENCE OF REGENERATION

In most cases when at operation a gap between the two ends of a severed nerve is found, there is, if sufficient time has elapsed, evidence of some degree of regeneration in the distal segment. This evidence has been found to consist of response to electrical stimuli of a nature not found in denervated muscle, rarely even of return of sensation, and still more rarely of return of motion. This so-called "spontaneous regeneration" led in World War I to the reopening of the theory of independent regeneration of the distal segment. However, recent investigators discovered evidence of growth of nerve fibers into the distal segment when the ends of the injured nerve were separated by a gap. Some had found their way from the end of the proximal segment; others were the result of branching of axones from the

uninjured part of the nerve above the level of injury; and still others resulted from branching from adjacent nerves. The observation of a large number of experimentally produced injuries, as well as of specimens removed at operation, afforded an opportunity to restudy the process of regeneration. Despite the numerous studies arising from World War II, at a considerable number of points there still exist differences of opinion. Indeed, much more work is necessary before there can be general agreement.

Some investigators have presented proof that the mechanism of cell and fiber advance is based on interfacial tensions between the protoplasmic surface layers and the contact surface in the medium. The orientation of Schwann cell or axone growth by oriented substrata, they reported, was produced experimentally by tension and selective enzymotic erosion in fibrous colloids and adhesive artificial fibers. Others attribute the orientation to a proliferation of mesodermal tissue.

The regeneration that occurs when the endoneural tubes are intact has been described as isomorphous. This may be found in nerve compressions, minor contusions, and other physical, possibly chemical, injuries. On the other hand, when the continuity of the endoneural tubes is interrupted, regardless of whether the organic continuity of the nerve is intact, heteromorphous neurotization occurs. This is to be found in severe contusions and nerve sections, and in grafts wherever the mesodermal endoneural tubes undergo necrosis, as in homogenous grafts. The essential histologic feature of heteromorphous neurotization is that fiber regeneration takes place in a new environment, and that the guiding elements for nerve regeneration as they persist in the secondary degeneration are disorganized or destroyed.

Although, as has been stated, it is extremely difficult to prevent regeneration of nerves, it often happens that an apparently adequate amount of regeneration of a nerve is not accompanied by the expected degree of recovery of motor function. That this may be due to failure of regeneration of muscle is self-evident. Reported studies show that there is strong and relatively early evidence of degeneration in human muscle, and the suggestive correlation between such changes and the advancement of fibrosis led to the belief that the former plays a considerable role in the latter. However, atrophy is the primary factor contributing to fibrous metaplasia in the muscle. There is a close correlation between atrophy and fibrosis in muscles at all stages and time intervals. The greater the time that has elapsed after injury and before operation, the greater is the mechanical fixation of the muscle by fibrosis.

The lessons learned in these and other studies have already proved to be of great value in the continued treatment of peripheral nerve lesions in military hospitals and those of the Veterans Administration. It is hoped that the records of the results obtained by treatment of those injured in World War II may be available and will lead to further answers to the questions posed to investigators.

EXPERIMENTAL STUDIES ON CONCUSSION

WILLIAM F. WINDLE

CONCUSSION is a disorder resulting when adequate mechanical force is applied to the skull; it is a transient state of unconsciousness of instantaneous onset with impairment of reflexes, followed by retrograde amnesia. Concussion of the brain is distinct from lesser traumatic injuries that leave the patient only momentarily dazed, from contusion, and from hemorrhages. Until recently it was frequently confused with the latter two conditions or was sometimes held to be unaccompanied by structural alteration in the central nervous system. The belief was widespread that it was simply a functional disturbance. The most acceptable theory related to the mechanics of concussion holds that it involves sudden generation of negative pressure, with temporary cavitation in the body tissues suffering traumatic effects.

A study of brain concussion is an important factor of any investigation of closed head injuries. The incidence of such injuries in modern warfare was expected to be great enough to constitute a special problem. The lack of full understanding of the fundamental processes leading to the sequelae of closed head injuries led to a desire to establish a program of co-ordinated and co-operative research in this field. Nine projects were subsidized by the Office of Scientific Research and Development, and several other groups of investigators independently pursued studies of a related nature.

The objective of the program of study of concussion of the brain was essentially twofold. In the first place, it was essential to arrive at an understanding of the fundamental functional and structural changes in the nervous system following blows or blasts in the vicinity of the head. In the second place, it was of great importance to evaluate the psychological factors of post-concussion, including post-traumatic personality changes, retrograde amnesia, and post-traumatic headache. One of the major aims was to correlate the post-traumatic cerebral syndrome with the results of experimental physiological and histopathological investigations.

Early in the war the study of brain concussion was placed on a firm experimental basis by Denny-Brown and his colleagues. They considered in some detail the functional alterations produced by applying force to the cranium. During the succeeding years other investigators applied various

physiological technics in an attempt to understand the physiological basis of the phenomenon. It soon became clear from observations on respiration, blood pressure, and certain somatic reflex activities that moderate concussion is a paralysis of reflex activation of motor centers in the brain, while the centers themselves may even be stimulated. Severe concussion produces complete paralysis, recovery from which is gradual. Momentarily excitatory effects are sometimes seen immediately on application of force to the brain, especially in nonanesthetized animals, but the most striking phenomenon appears to be the paralysis of reflex mechanisms.

In studying brain concussion in animals one cannot apply all the criteria used to diagnose the condition in man. It is difficult to determine unconsciousness — to say nothing of amnesia. Abolition of such reflexes as the corneal reflex and interruption of respiration signify the occurrence of concussion in experimental animals. The transient nature of these and other paralytic symptoms is an essential feature of concussion. Differential susceptibility of various groups of neurons was observed while studying the effect of electrical stimulation by means of electrodes implanted in the brain by the Horsley-Clarke technic. Interneuron systems of the brain stem were more readily affected than primary sensory or primary motor neurons. The threshold increase and threshold recovery time were roughly proportional to the intensity of the concussion. The threshold increase was sudden and rapid; the threshold recovery time, relatively gradual. Such differential functional alteration in the brain during concussion is an essential feature of this phenomenon. Somewhat similar observations were made on the spinal cord after blows applied to the back.

Other experiments were performed in an effort to demonstrate a structural counterpart of the functional manifestations of concussion. Here, too, the arbitrary criterion of concussion was abolition of the corneal reflex and interruption of respiration. Concussions uncomplicated by hemorrhage, skull fracture, or contusion were produced in animals. Exacting physiological and histologic controls were set up.

In one group of experiments the heart was cannulated for perfusion before concussion and the blow was struck eight to thirty seconds before the fixing fluid reached the brain. Significant alterations in the Nissl bodies of certain neurons were observed. Subtle changes in the larger nerve cells of the brain stem corresponded to more pronounced changes in the same neurons in other animals permitted to live after the head was struck. Chromatolysis was demonstrated less than twenty-four hours after concussion. The changes reached a maximum intensity by the sixth or eighth day.

Not all nerve cells were equally affected by concussion. Primary afferent and primary efferent neurons of the cranial ganglia and cranial nerve nuclei were practically unaffected. All concussive blows induced changes in the large cells of vestibular nuclei and the scattered large neurons of the brain

stem tegmentum. With increasing severity of concussion, the red nuclei, the nuclei of the trigeminal spinal tract, and the cerebellar and cochlear nuclei became involved. The amount of damage was proportional to the strength and number of blows struck. While it was easy to see at a glance the effect of concussion on larger nerve cells of the brain, closer examination of sections through the brain stem revealed that great numbers of medium-sized and small nerve cells were at least as much affected. Some parts of the brain appeared to be highly resistant to concussion. No changes were observed in the basal ganglia or the cerebellar cortex. The latter did not escape entirely, but the changes differed from those seen in the brain stem, and chromatolysis did not develop to the extent observed in several species of animals.

An important clinical concept of concussion is that the phenomena are reversible and that damage is not permanent. Recent studies have shown that this is not strictly true. Reduction in the number of large interneurons throughout the brain was observed by careful enumeration of the cells in certain nuclei in animals that had received simple, uncomplicated concussions at weekly intervals. Comparisons were made with controlled specimens. The series of blows appeared to have a cumulative effect. After two light and four severe concussions, each made approximately a week apart, the large interneurons of the reticular formation of the brain stem, as well as the large neurons of the lateral vestibular nuclei, were reduced in number to nearly half that found in the control. No significant loss was encountered in the motor nuclei of the trigeminal nerve. In view of these experiments, there can be no doubt that concussion of the brain can lead to destructive changes in some nerve cells that are not reversible — that permanent cell loss can occur after concussion.

Not only were changes in nerve cells demonstrable during and after concussion, but damage was found in fiber tracts in certain parts of the brain. The changes were focal in nature and did not involve the tracts over their entirety. In the early stages after severe blows the changes were most marked in the central part of the cerebellum and in the peripheral pathways of the medulla oblongata. There was no relation between the occurrence of hemorrhages and the damaging of myelin sheaths. The degeneration of the type observed here was not secondary to loss of nerve cells but appeared to be a primary result of the concussive blow. It is interesting to note that the groups of nerve fibers whose threshold to electrical stimulation was found to be practically unaltered after concussion showed less degeneration than did other fiber tracts of the brain.

The ravages of concussion were compared with changes in the nervous system after sectioning nerve fibers and after anoxia, edema, and other factors. The histopathological picture was significantly different in these latter conditions. The changes in concussion were not the result of hemorrhage or skull fracture. Concussive blows that failed to produce hemor-

rhage did not render blood-vessel walls permeable to vital dye substances. Edema of the brain was only slight in simple concussion, and it was demonstrated that the cytopathology was unrelated to this.

One group of investigators studied the effect of underwater blast. The animals were submerged in water in a large U tube. Impacts were produced by dropping a weight on a steel plate in contact with the water at the other end of the tube. The effects on the animals were similar to those of explosives in air or water. Fatal pulmonary hemorrhages and perforation of the intestine occurred. When the animals were completely submerged, impacts somewhat greater than those producing the pulmonary hemorrhages resulted in disruption of the walls of the air-containing sinuses of the head. The immediate manifestations were nosebleed, interference with respiration, and, at times, disturbances of equilibration. However, no symptoms of brain concussion developed. As a matter of fact, concussion resulted only when forces were used that were fifteen or more times as strong as the minimal lethal forces applied to the whole animal. This was determined by placing only the top of the animal's head in the water at the time of the blast. It thus appears that brain concussion is not to be considered as an effect of blast injury. If the blast is strong enough to produce concussion, it most certainly will lead to sudden death.

The brain concussion generated by intense force of underwater blast applied only to the head resulted in changes in nerve cells of the brain. The damaged cells were scattered throughout the brain stem, much as they were in animals that had received mechanical blows on the head.

Study of the electroencephalogram in concussion of experimental animals was carried out. Application of sufficient impact was accompanied by the loss of corneal reflexes and other symptoms. The electroencephalogram showed an immediate alteration from the previously normal pattern, beginning with a marked but brief pattern suggesting overactivity. With lighter impact there might appear low-amplitude, fast waves on a nearly flat baseline, suggesting activation. With stronger impacts the tracing was flat and smooth. The duration of such changes varied with the depth of concussion, and the original electroencephalogram pattern was gradually restored as reflexes became normal. Several periods of concussion could be experienced by one animal without permanent alteration of the electroencephalogram.

Such experimental results as these raised the question whether the electroencephalogram can be considered a useful diagnostic aid in simple concussion. At the initial stage of unconsciousness during which reflex function was impaired one could expect alterations in the record, but later on little change might appear. This has been borne out by studies conducted on human patients.

Only a few of the experiments in brain concussion have been dealt with. There are other aspects of the concussion problem that were investigated

under the Committee on Medical Research and that lack of space prevents considering. Details concerning these will be found in many of the articles listed in the Bibliography. Many of the data gathered during the months of intensive experimentation have not as yet been fully evaluated, and it will take several years to complete the picture outlined during the rush of wartime.

A second major division of the concussion problem was the investigation of psychological disturbances by experimental methods. Several groups of investigators undertook intensive study of human subjects; others employed experimental animals.

Significant observations were made in experiments on the effects of concussion on learning and memory. Using a simple maze (Fig. 1) the animals
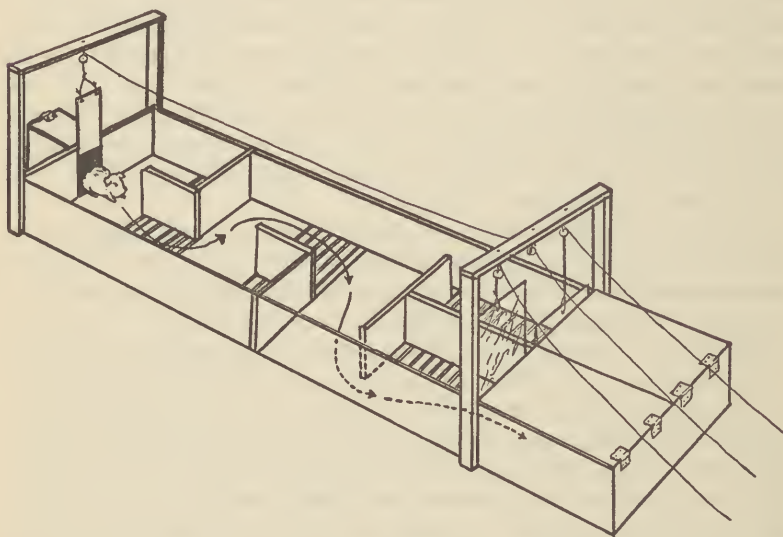
FIGURE 1. *Maze used in observing effects of concussion on learning and memory.*

learned (primarily with visual cues) that the correct exit lay behind the first blind alley, regardless of which side this was on. Electrical grids in the floor of the alleys and in the floor of the terminal pathway provided punishment for incorrect running. Food, water, and darkness provided rewards for correct solution of the problem. Once an animal had learned the problem with the first alley on the left, the alleys were shifted to the opposite side. When the new situation had been mastered, the problem was alternated, left and right. The investigator who performed the maze experiments

was not told by the investigator who produced the concussion which were the experimental and which the control animals.

A number of guinea pigs were selected and approximately half of them were subjected to concussion. They were taught the problem for the first time six days, thirty days, or ninety days later. Control animals learned it more readily, with the production of fewer errors and with fewer repetitions of errors, than did the experimental animals. At six days after the concussion the difference between the performance of the control and the experimental animals was significantly great. At thirty days the difference was still significant, although not as marked as at six days. At ninety days the difference was insignificant. Only the initial learning after concussion was significantly impaired.

In another experiment animals were taught the problem until they had become letter-perfect. Six days later a retest demonstrated perfect retention. A second investigator then took all the animals and arbitrarily selected certain ones for concussion. Six days after concussion the animals were returned to the maze. At that time it was found that all the control animals executed the problem perfectly. Each animal that had received concussion was correctly picked out by the testers because it failed to obtain a perfect retention score. This experiment was repeated using thirty-day and sixty-day intervals following concussion. Memory was still significantly deficient after the longer interval.

In another experiment, memory of a problem learned after concussion was investigated. The control animals were always superior to the experimental animals and made significantly fewer errors at each retesting. In animals trained six days after concussion retention was significantly better if the retest interval was as short as a week or ten days. When the interval between tests was lengthened too much, retention became progressively poorer. In animals trained thirty days after concussion the longer intervals — one to two months — had less effect on retention than they had in the previous group. Apparently, then, if a problem is learned after a significant interval is allowed for recovery, it will be retained with less loss than when the problem was learned too soon after the accident, but in any event retention is never as good as in the uninjured animal.

When all the data from experiments in animals have been assembled and analyzed, it will be of great interest to correlate the results with those noted by several groups of investigators who used human subjects. Such human studies led to the discovery of little permanent effect of simple brain concussion. Prolonged disability resulting from civilian head injuries was related with symptoms of a purely psychiatric nature, especially anxiety neurosis. The personality of the patient was found to color this neurosis, but statistical studies did not indicate a basis for the neurosis in personality factors. Post-traumatic anxiety remained the greatest single cause of disability.

Performance tests gave some indication of immediate prognosis when given during the period of hospital admission, but when these and intelligence tests were done at a first follow-up or later, they proved to be of minimal value in estimating further disability. The extremely detrimental effect that alcoholism has on the phenomena of head injuries and their recovery was clearly demonstrated.

Cerebral concussion is a phenomenon that not only has important practical considerations but also merits attention as a fundamental mode of reaction of nervous tissue. Although recent investigation has done much to clarify its essential nature, there are many aspects of the subject that present unsolved problems. Some of the factors that determine the presence or absence of concussion in penetrating gunshot injuries of the brain are now explained. The separation of the effects of concussion and of multiple hemorrhages and the better understanding of the pulmonary lesion due to explosive violence have seemed to clear up many of the doubtful features of the effects of explosive blasts.

The introduction of methods of reproducing standard degrees of cerebral concussion and of measuring both the quantity of injury inflicted and the amount of effect produced have opened the possibility of measuring the relation of concussion to nervous metabolism. The effect of concussion on synaptic transmission and on the gradual development of histologic changes that follow the immediate structural disturbance are at present complete enigmas, which invite investigation. The relation of concussion to shock, to fatigue, and to the changes induced by anoxia, acidosis, electrical shock, and so forth has been incompletely explored.

In all investigations on these subjects, the character of disturbance of nervous activity should be closely defined in order to avoid the confusion that has arisen and that will still occur if traumatic concussion is not clearly differentiated from other kinds of disturbances of nervous activity. In some respects it is necessary to keep the different kinds of mechanisms of production of damage clearly identified.

With the rapid increase in knowledge of concussion in recent years, it is a question of whether a more accurate definition of cerebral concussion can now be devised. Because the condition is primarily of clinical interest, it is unlikely that any definition not based on the disturbance of consciousness or awareness would be generally acceptable. Although it is possible that some degree of excitation of nervous tissues can occur on application of force to the skull, it is necessary to emphasize the loss of nervous function that occurs, at least momentarily, and that is the essential characteristic of brain concussion. A physiological definition should also take into account the occurrence of a similar and probably identical phenomenon in the spinal cord. It now has to admit histologic change but not necessarily vascular or hemorrhagic factors. It is possible to say definitely that some nerve cells are

completely destroyed as the result of brain concussion. We do not yet know what proportions of what groups of nerve cells need be irreparably damaged to bring about the symptomatology of the post-concussion syndrome.

With these considerations in mind, it is suggested that concussion can be defined as a transitory and partially reversible nervous reaction with immediate onset following physical stress of sufficient violence and brevity, and characterized by progressive, although not necessarily complete, recovery. In man amnesia, both retrograde and anterograde, is the chief external sign, accompanied by a loss of reactivity, which is its clearest feature in animals. The loss of reactivity affects all tissues subjected to such stress, but the duration of paralysis varies with the complexity of the nervous factors as well as with the intensity of injury.

CHAPTER XV

FROSTBITE AND TRENCH FOOT

J. M. CRISMON

THE AVAILABLE information on the incidence of cold injuries in World War II indicates that little had been accomplished since World War I in the development of effective methods of preventing initial injury or subsequent gangrene. The Finnish Army reported over 5000 cases of frostbite in January 1939, and President Kalinin estimated that 2,000,000 to 3,000,000 German soldiers in Russia were incapacitated by cold during the winter of 1942–1943. During World War I the French Army reported trench foot as accounting for 3.02 per cent of their battle casualties, and up to December 10, 1944, the incidence was 3.0 per cent, a figure comparable to that reported from United States forces. In 1944, following the November offensive and again after the German counteroffensive in December, the incidence of cold injury increased at an alarming rate. Figures from the armies on the Continent show that for trench foot alone the ratio of cold injury to battle casualties was 16.8:100 in November and a little over 30:100 in December.

The methods of treatment of the acute stages of cold injury accepted by British and American military surgeons up to 1944 were in general conservative. Prompt thawing at moderate temperatures was regarded as essential, but the later management of frostbite as well as immersion foot and trench foot involved the maintenance of the injured extremity in a cool, dry environment, either exposed to the air or covered with sterile dressings. In the absence of infection, amputation was delayed until drying of the gangrenous parts was complete and separation had begun. In the chronic stages, characterized by fibrosis, neuropathies, disturbances of the peripheral circulation, and sweating, treatment consisted mainly of physical therapeutic measures directed toward improvement of blood flow and restoration of muscle and joint function.

In August 1944, a group of investigators at Leland Stanford University began to study the prevention of gangrene following frostbite. Since the effects of cold on mammalian tissues, in the absence of physical disruption of cells, are largely reversible, it seemed probable that the development of gangrene in regions exposed to severe cold is the result of secondary changes culminating in the arrest of blood flow and ischemic necrosis.

The pathologic and physiological reactions to severe cold injury are essentially similar to those seen in tissues reacting to superficial thermal burns. It was hoped that the control of swelling, effective in burn treatment, and the maintenance of low tissue temperatures, shown to retard the development of gangrene in ischemic extremities, might be combined effectively to prevent gangrene following cold injury. Various therapeutic measures were tested on frostbitten feet and ears of rabbits, in which the injury produced by immersion in cold liquid was known to be severe enough to produce gangrene and complete loss of exposed tissue in all the untreated controls.

The course of events following thawing and the return of blood is characterized by an initial vascular spasm, subsequent hyperemia, and massive edema. Direct observation of skin capillaries in the ears of rabbits shows that within ten minutes after thawing most of them have become filled with packed red cells; flow is arrested and stasis is complete. In a few vessels, blood flow persists and is more rapid than that observed before injury. The latter vessels include arteriovenous anastomoses and a few capillaries affording more or less direct communication between arterioles and venules. This rapid blood flow accounts for the relatively high skin temperatures observed in the frostbitten parts during the first few hours after thawing. Subsequent measurements of skin temperature and tests with fluorescein by Lange's method show that some blood flow persists as long as fifty hours after injury. It is possible that the reactions of the rabbit ear are not altogether representative of the reactions of the frozen extremities.

Neither slow thawing of frostbitten parts nor their maintenance at low temperatures after thawing was effective in preventing gangrene. On the other hand, immediate and rapid warming of frozen ears by immersing them in water at 42° C. resulted in the saving of 80 to 100 per cent of the injured tissue. Frozen feet when treated in the same manner developed gangrene only in the toes following degrees of injury that usually produced loss of tissue up to the line of immersion at the level of the tuberosity of the fifth metatarsal bone. These observations are in agreement with those published by the Russian worker Ariev. While the result in terms of tissue saved was favorable, this treatment was followed by marked induration and ultimate massive fibrosis in the surviving tissue.

None of the vasodilator drugs produced reversal of the process of injury. Paralysis of the sympathetic nerve supply to the ear by procaine block of the stellate ganglion delayed the onset of stasis until edema was well advanced, but did no more than delay the onset of gangrene by about one day.

Of the various methods used to prevent or control edema, only the application of pulsating pressure was a complete failure. It was hoped that the rhythmic application of positive pressure about the injured part would facilitate both blood flow and lymphatic drainage.

Closed, nondistensible dressings applied to frozen feet were found to

produce about the same saving of tissue as that accomplished by rapid warming. These dressings consisted of casts made with ordinary plaster or with layers of gauze bandage painted with plastic resins dissolved in volatile solvent. Successful use of this method was possible only when the cast was applied before marked swelling had occurred. Careful padding, preferably with thin layers of lamb's wool, was an important point of technic. Incorporation of thermocouples in contact with the skin of the toes permitted frequent measurements of skin temperature without disturbing the dressing. Unfavorable response to treatment, possibly owing to uneven distribution of pressure, was indicated by an early fall of toe temperature. The best results were obtained when the dressings were left on for about seven days; their removal as early as the fourth day was followed by edema and greater loss of tissue. There was an abundant growth of hair on the surviving tissues following removal of the casts, and no signs of fibrosis were detectable.

Pressure dressings of elastic webbing applied over massive padding with lamb's wool afforded approximately the same amount of protection against gangrene as did rigid casts and rapid warming. Fibrosis in surviving tissues was slight to moderate in amount and varied with the interval between thawing and the application of the pressure dressing. From the point of view of practical application, pressure dressings should be of much greater value than rigid dressings, since the latter must be applied prior to swelling. Few cases are encountered in ground forces in which frostbite casualties receive treatment before swelling is well advanced. As a first-aid measure in high-altitude frostbite or in frostbite occurring in naval personnel, rigid dressings might be useful in preventing subsequent gangrene. However, this method requires further investigation under practical military conditions. It is proper to point out that not all investigators have been uniformly successful in demonstrating salvage of tissue through the use of pressure dressings. Variations in technic may account for varying results.

Since blood flow in the injured tissues has been observed to decrease with time after injury, early treatment is essential. It is considered unlikely that any of the above measures would be successful if applied later than the first six to twelve hours after thawing.

The second study of the prevention of gangrene subsequent to frostbite and immersion foot was that conducted at the New York Medical College. The investigation, which began in April 1945, was subsequently continued as a project of the Surgeon General's Office.

Largely through the development and application of the fluorescein test in earlier studies on frostbite, adequate proof was obtained that good blood flow existed through severely frostbitten tissue of rabbits for at least six hours after thawing. In volunteers and in one actual case of severe frostbite, evidence was obtained that the blood flow might persist for as long as forty-eight hours

in man. Histologic examination of tissues obtained by biopsy and after sacri-
fice of experimental animals showed that the organization, by fibrin forma-
tion, of trapped red cells and the protein-containing exudate outside capil-
laries did not occur for about seventy-two hours. In view of these favorable
time relations, it seemed profitable to attempt the prevention of clotting by
use of the anticoagulant heparin.

In the experiments on volunteers, it was possible to show that the fluid in
frostbite blisters has almost the same protein content as plasma. This blister
fluid, which corresponds to the interstitial fluid that causes the swelling in
the exposed parts, clots within forty-eight hours, whereas it stays liquid
throughout if the patient is heparinized. This makes possible resorption of
the edema without deposition of fibrin and collagenous replacement.

Tests of the response to heparin therapy were carried out on frostbite
produced in the tail and legs of rats, the feet and abdominal skin of rabbits,
and frostbitten skin areas in human subjects. The injuries were sufficient to
produce gangrene after about seven days in all cases in which no treatment
was used. Adequate heparinization was found able to prevent thrombosis
and subsequent gangrene. Favorable results were obtained with heparin
only when its use was begun as early as the stage of hyperemia following in-
jury and when it was continued at optimum dosage for eight to ten days.
The animal experiments indicate that the red-cell sludge that forms in the
nonheparinized animal owing to plasma loss does not organize for at least
three days. It does not organize at all if heparin is present in the blood
stream. Experiments in volunteers indicate that an interval of forty-eight
hours between the end of the exposure and the start of treatment reduces the
success only slightly, although the earliest possible moment should be chosen
for beginning the therapy.

Even brief returns to clotting times shorter than thirty to sixty minutes
jeopardized the success of treatment. The dosage required in rabbits was
50 mg. every twelve hours by vein. Attempts to obtain a slow, uniform
absorption of heparin into the blood by administering it subcutaneously in
Pitkin's menstruum were complicated by bleeding into the injection site.
The best method of administration was that of continuous intravenous drip.

Infection in the previously frozen area was a serious complication. It is
interesting to note that tightly applied sterile dressings from the outset and
reapplication under sterile conditions, as employed successfully in the treat-
ment of burns, solved this problem.

Experiments on human subjects demonstrated that, with adequate heparin-
ization, injury to the tissues was confined to blistering, whereas in un-
treated controls necrosis of the exposed area developed.

Brief trials of the use of heparin were made by the group at Stanford
University, and a few experiments with casts, pressure dressings, and rapid

warming were done by Lange and his co-workers. In each case the results were unsuccessful. Neither series of experiments was large enough to justify more than the suggestion that unfamiliarity with the technics accounted for the disparate results. Further studies of combinations of treatment involving heparinization and control of swelling with casts or pressure dressings are needed.

Results from both these projects emphasize the following points. The process of injury from frostbite is, within certain limits, reversible. Injury sufficient to produce gangrene does not occur at the moment of exposure, but depends on progressive reduction of blood flow in the exposed parts many hours after thawing. Lastly, possibility of success in the prevention of gangrene diminishes rapidly with increasing length of time between exposure and the beginning of treatment. Therefore, adequate materials and instruction should be provided to put the recommended methods of treatment in use as first-aid measures in severe cold injury.

A third study was begun in May 1945, at Tulane Medical School for the purpose of developing objective tests that would demonstrate the existence of active inflammatory processes and aid in evaluating the degree of severity in mild chronic trench foot. The problem was attacked by comparing measurements of skin temperature in patients with uncomplicated chronic trench foot with those in normal control subjects under prescribed conditions of environmental temperature, humidity, amount of clothing, and diet. Data were obtained before occlusion of blood flow to the foot, during occlusion, and during the subsequent reactive hyperemia. These observations were supplemented by quasi-objective evaluations of skin color and the rate of refilling of skin blood vessels emptied by manual pressure. The comparative rates of water loss from the skin of the feet in the two groups were measured and found not to differ significantly.

In general, the initial toe temperature is higher, the rise during reactive hyperemia is greater, and the time for refilling of skin blood vessels after compression is shorter in patients with clinically active trench foot than in normal or psychoneurotic subjects. There was a high correlation between the clinical severity of the disease and the degree of deviation of values measured in patients with trench foot from those obtained in normal subjects.

According to these investigators, the number of patients employed in this study (108 normal controls, 51 psychoneurotic controls, and 49 patients with trench foot) does not justify expression of the amount of inflammatory activity in numerical terms. They suggest, however, that an index that might appropriately be called the "Activity Index," based on the factors of initial stabilization temperature, number of degrees of fall in temperature following fifteen minutes of occlusion, and number of degrees of rise during hyperemia after the release of the circulation, can be devised to express the amount of inflammatory activity in a part injured by cold and moisture.

The desirability of obtaining objective, quantitative means of assessing the state of the disease in chronic trench foot is obvious. Whether or not the study of a larger group of patients would yield data permitting the use of a single numerical expression for evaluating individual cases, such a study is warranted to increase the reliability of the various separate parts of the test.

NEW SURGICAL PLASTICS AND HEMOSTATICS

VIRGINIA KNEELAND FRANTZ

THE SEARCH for new plastics to be used in special surgery and for new methods of hemostasis in general as well as special fields was not initiated by the military needs of the recent conflict. Work in progress, however, was intensified and accelerated as these needs became apparent. Repair of tendons and nerves, reconstruction of joints, and staunching of hemorrhage were not new problems. They were, however, problems which had not been solved before the onset of hostilities and for which even a partial answer might benefit large numbers of casualties. Additional research and laboratory personnel supplied by funds from the Office of Scientific Research and Development and the challenge of war greatly expanded studies begun on a small scale and at a leisurely pace.

As is often the case in any field of medical investigation, similar projects had already been undertaken independently. The emphasis had been on some particular phase of interest to the individual or group in the laboratory or clinic. This happy natural phenomenon of what might be called multi-centric origin of ideas constitutes both an incentive and a check for the resulting discovery. Another common experience is that the search for a new method may lead to the finding of materials or technics applicable in a wholly unrelated field, so that a strictly chronological account is well-nigh impossible. This has been the story of the development of the new absorbable nonirritating materials for use in surgery, their destiny being to supplement, it should always be emphasized, the older established technic, and to be used only where the old has failed or the new has proved beyond peradventure immediately more effective and equally without hazard.

The impetus for the development of new methods of hemostasis came, as might have been expected, long before the war, from the needs of neurosurgeons. Where traditional methods — clamp, ligature, and suture — are impractical, the value of the clot is obvious. Muscle strips to supply additional clotting enzyme having been proposed as early as 1911 by Cushing, the next significant advance along this line was reported in 1938 by Seegers and his associates — the purification of thrombin from beef plasma. Subsequently, as a result of the large-scale study of the fractionation of human

plasma [1] undertaken in the Department of Physical Chemistry at Harvard University, human thrombin became available. This water-soluble enzyme, both bovine and human, was effective in controlling surface ooze when used alone as a spray. In spite of the possibility that bovine thrombin might have antigenic properties for human beings, extended clinical trial has failed to demonstrate any such hazard. It was immediately adopted by neurosurgeons and used not only as a hemostatic spray but to wet the traditional cotton sponges, which had formerly been wetted with saline solution and held with gentle pressure against small bleeders.

Even with the accelerated clotting so achieved, however, removal of the sponge was often followed by renewed bleeding. It therefore became clear to a number of different investigators that a carrier of thrombin that could be left in place would be highly useful. The first of these again came from human blood, collected by the Red Cross and fractionated at Harvard. The fibrin sponge — "foam," as it is aptly designated — was processed under sterile conditions to form a dry, porous, brittle, cream-colored material, which was found, after extensive experimental trial, chiefly in monkeys, to be nonantigenic, nonirritating, and absorbable in the tissue with a minimum of inflammatory response and of residual scar. When soaked in thrombin solution it was an ideal application for surface bleeding, and it was immediately put to use in neurosurgery and subsequently advocated for control of hemorrhage in other fields. It was packaged in time for use as early as the final phase of the African campaign, and was widely distributed to Army, Navy, and civilian hospitals here and in Great Britain for clinical trial.

At Columbia University another absorbable material was being studied, not at first with any idea of its possible use as a hemostatic, but particularly as a film for the prevention of adhesions in the repair of tendons in a sheath. This was cellulose — in the form of paper and cotton — which had been oxidized by nitrogen dioxide, a process that resulted in the formation of carboxyl groups, thus changing the cellulose into an organic acid, now designated by the chemists in the Organic Research Laboratories of the Eastman Kodak Company, where it was developed, as "celluronic acid." According to the degree of oxidation the carboxyl content could be varied, and with this the physical and chemical properties of the material. After preliminary trials with animal implants, a standard was set that the carboxyl content should be between 17 and 21 per cent and that the material should be soluble in 0.15 M solution of sodium bicarbonate. Investigation at Columbia was at about this stage when fibrin foam was suggested as a carrier of thrombin. It was thought that oxidized cotton might serve as well as fibrin foam and that this material deserved more intensive study under OSRD(CMR) contract, which was accordingly undertaken.

[1] See Chapter XXVIII.

Oxidized cellulose in another physical form — that is, gauze — was immediately envisaged as a possible solution to the difficulties encountered in general surgery. This was a revolutionary thought to those who had spent years preaching the dangers of foreign bodies in tissues. With some trepidation, therefore, oxidized gauze was also obtained, and extensive animal studies were begun to determine whether it could be used safely as packing and left in the clean closed wound. This was unprecedented, and it was with great skepticism that bleeding from large lacerations of the liver, kidney, and spleen was staunched with what appeared to be an ordinary gauze sponge, this being left in situ. Moreover, it was with special misgivings that large amounts of this gauze were left in the free peritoneal cavity, where similar surgical accidents in the past had led to such disastrous complications. No particular courage was required to leave processed human fibrin in a wound that was bound to be filled by this material from the patient's own plasma, but it was with a deep sense of guilt that one closed the abdominal wall, even in the experimental animal, knowing that there was a gauze sponge in the pelvis. When, on later exploration, it was found that the oxidized gauze had been absorbed without damage to the tissues, this seemed miracle enough. Gauze packing had traditionally been used as a control for bleeding that could not be stopped otherwise, and to have it absorbable was almost ideal. In checking, however, to see whether there was any advantage in the use of thrombin with the gauze (and finding that they were mutually incompatible), it was appreciated that the gauze had a specific hemostatic action in its own right. Owing apparently to its acidity, which makes it combine with hemoglobin to form a salt, the oxidized gauze stopped bleeding more effectively than ordinary gauze. It rapidly turned black in the wound, became sticky, and filled the wound space evenly and closely. It was then an obvious possibility that, in the contaminated wound, the gauze might be impregnated with antibiotics and thus serve two purposes. The only antibiotic tested so far in combination with oxidized gauze has been penicillin, and the acidity of the gauze in solution is such as to inactivate this drug and thus to make the combination impractical.

There have, then, been developed two absorbable hemostatic packings that can with impunity be left in the tissues of a closed wound. Many other substances have been suggested, including sponges made of starch, casein, nucleoprotein, and so forth. Of those studied in controlled experimental and clinical series, much the most promising one is made of gelatin, devised as a cheaper and more easily available material than human fibrin foam and bearing the appropriate trade name Gelfoam. This, like fibrin foam and oxidized cellulose, is nonirritating, apparently nonantigenic, and absorbable. It was originally suggested as a carrier of thrombin, but has also been used without the enzyme. All these materials have now had extended clinical trial, but as yet there have not been enough clinical comparisons by un-

prejudiced observers to determine the relative merits and possible different fields of usefulness. This remains to be done.

In the search for useful surgical plastics these same substances and others were also investigated. Plasma again comes to the fore in reconstructive surgery of nerves, this time in the form of autologous clot for better apposition of nerve ends. Another animal protein, nonantigenic and absorbable, suggested for nerve splicing and bridging of nerve gaps is collagen, prepared in the form of elastic plain tubes. This was compared with tantalum cuffs and with frozen dried artery and nerve grafts. Nonabsorbable fibers for bridging tested in tissue culture and in vivo are rayon, cellulose, acetate, nylon, cordura, and glass. These plastics are discussed in detail in the chapter on repair of peripheral nerve injuries.[2]

For prevention of adhesions many films have been suggested. Those particularly studied have been fibrin, collagen, and oxidized cellulose. These are for the most part still in the stage of laboratory investigation, although a few clinical trials have been made.

Oxidized cellulose, because of the acidity of the material, was shown to have an inhibitory effect on the formation of early callus in bone. This property was the basis of experimental work in the formation of pseudoarthroses with controls in the same animal of fibrin foam, gelatin sponge, and no foreign material. The false joints thus obtained with oxidized cellulose seemed sufficiently promising to warrant clinical trial of the material in arthroplasty for bony ankylosis, and this is now under way.

It has been emphasized by all investigators in the fields of surgical plastics and absorbable hemostatic materials that these are not to replace any successful method that does not call for the introduction of foreign bodies other than the traditional suture and ligature. Nor are these suggested to save the surgeon time, or the patient hours of anesthesia and operation. Even if the foreign material introduced is absorbable, it should only be used in bulk in surgical procedures that might otherwise be impractical, such as lobectomy of the liver. Moreover, when this is done, for a good reason, it must be borne in mind that the surgical risk is somewhat increased, even if the material is shown to be nonirritating, because contamination that might otherwise be overcome by the tissues of the host may result in infection in the presence of the foreign body before it is absorbed.

[2] See Chapter XIII.

CHAPTER XVII

IMPROVEMENTS IN X–RAY DEVICES

PAUL C. HODGES

$\mathbf{P}$HOTOELECTRIC exposure meters have been widely used in ordinary photography in recent years, but although the need for them is even greater in the making of clinical x-ray films, they have not heretofore been used for that work because of certain technical difficulties. Prominent among these is the fact that phototubes are not directly sensitive to x-rays but must be activated by the fluorescent light given off when certain chemicals known as phosphors are subjected to these rays. The visible light emitted by such phosphors is so feeble that the phototube current induced by it must be amplified before it can be read conveniently, and special precautions are required to assure parallelism between the response of x-ray films and that of such phosphor-phototube systems to the action of x-rays over a reasonable range of working conditions.

It is important in clinical radiology that x-ray films be of approximately uniform density regardless of the thickness and radiopacity of the part that is being examined. All else being equal, density is determined by the duration and intensity of the x-ray beam relative to the thickness and radiopacity of the part. The commonest practice is to hold intensity constant and to vary exposure time for varying thickness and opacity. X-ray machine manufacturers have produced dependable motor-driven timers, some of which are accurate to an exposure time as short as 1/120 second, but the measurement of part thickness is not as easy as one might suppose, and even the most skilful and experienced technician is not always able to estimate accurately the radiopacity of the part being examined.

Dr. R. H. Morgan, acquainted with the meagre literature on the subject and convinced that modern developments in electronic tubes and circuits held promise of success in this field, built some exposure meters that employed simple (R.C.A. 929) phototubes connected to external amplifiers and to mirror-type microammeters, and published a description of them. These meters, although successful in the laboratory, were not sufficiently rugged for use by the armed services, but with the introduction of the Radio Corporation of America's series of multiplier phototubes (R.C.A. 931, 931A, and 1P21) the way was paved for simpler and more rugged instruments.

The objectives of our project were to develop x-ray exposure meters for

gauging accurately the thickness and radiopacity of any part of a patient's body, and if this phase was successful to substitute electronic relays for the meters, thus converting the instruments into photoelectric timers that would be capable of integrating exposure time, beam intensity, part thickness, and part opacity, automatically shutting off the x-ray machine when the film had received exactly the proper amount of radiation. It was recognized that the simplest application of the phototimer and the one currently most valuable to the armed services would be for the automatic timing of 35-mm. and 4-by-5-inch microfilms of the chests of inductees. Adaptation to general radiography would be more difficult and was less likely to be completed in time to assist in the prosecution of the war.

The experimental laboratory and shop of the Division of Roentgenology of the University of Chicago were used for the developmental work, and the preliminary testing was done in the x-ray laboratories of that institution. Subsequent field testing took place in certain laboratories and hospitals of the Army, Navy, and United States Public Health Service.

RESULTS

EXPOSURE METER

Six x-ray exposure meters (Fig. 2) were completed, two being turned over to the Army, two to the Navy, and two to the University of Chicago, which subsequently purchased them. The instrument consists of two parts. The detector, a small lightproof metal box with a bakelite cover, contains a fluorescent screen, a 931 multiplier phototube, and a resistor network for the tube. The meter unit contains a microammeter calibrated in seconds, filament transformers, a high-voltage direct-current source, and a potentiometer for the empirical calibration of the instrument. To use the exposure meter one places the detector beneath the part that is to be examined, plugs the detector cable into the meter unit, makes a time exposure, and then reads on the meter the exposure time that will be required to ensure a film of standard density. The instrument is portable and is not connected to the x-ray machine in any way, but it does have the disadvantages of requiring a preliminary trial exposure, and assurance that the time switch is accurate and that the intensity of the beam is the same when the film is being made as it was during the trial exposure.

PHOTOTIMERS

In the phototimer for photofluorography (Fig. 3), the detector unit requires no fluorescent screen, since the phototube receives light from the same screen that is being photographed. The control chassis that replaces the meter

unit contains among other things a gas thyratron (R.C.A. 1C21), a metering condenser, and a small, quick-acting, normally closed relay. The x-ray exposure is initiated manually and terminates automatically when the current from the phototube has charged the metering condenser to a critical point. When this point is reached the thyratron fires, thus activating the
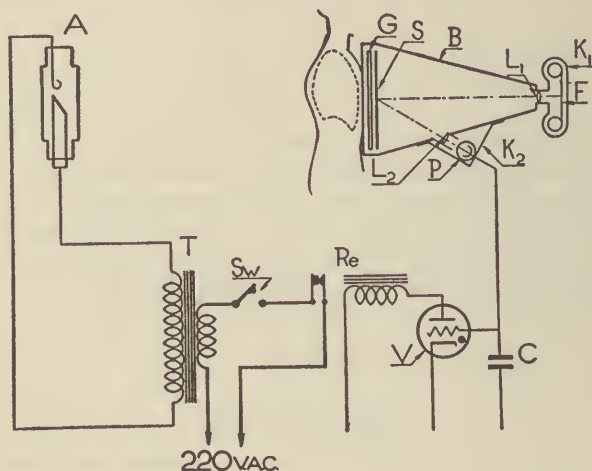
FIGURE 3. *Phototimer for photofluorography (simplified diagram).*
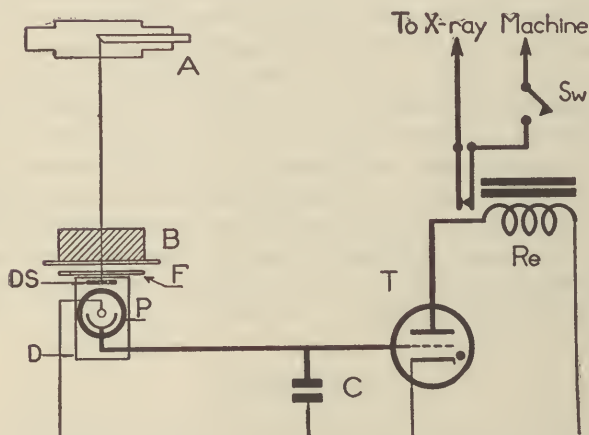
FIGURE 4. *Phototimer for general radiography (simplified diagram).*
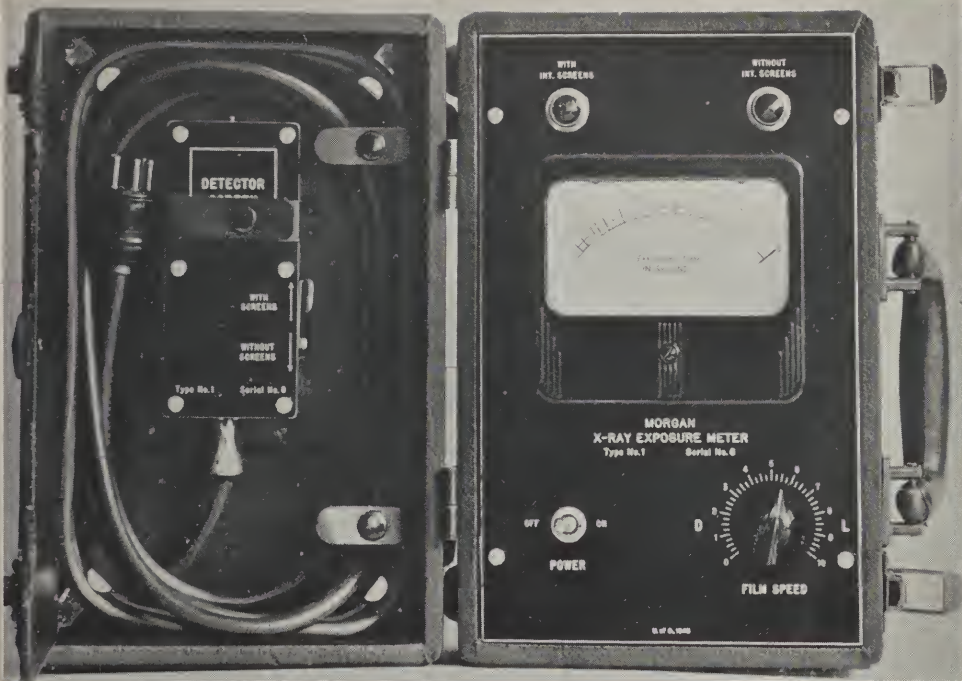
FIGURE 2. Morgan x-ray exposure meter.
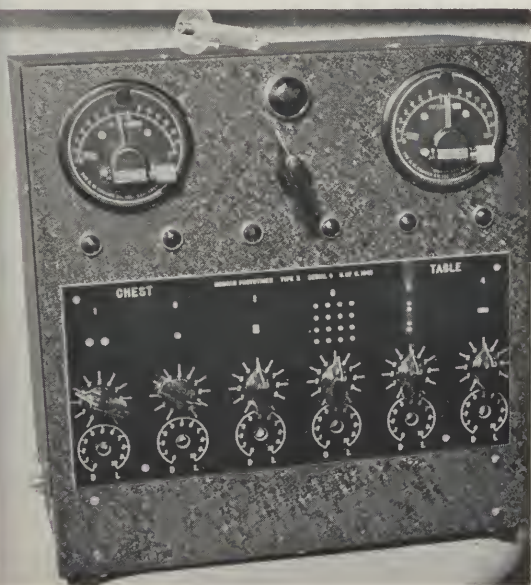
FIGURE 5. Phototimer for general radiography as supplied to the Army and Navy.
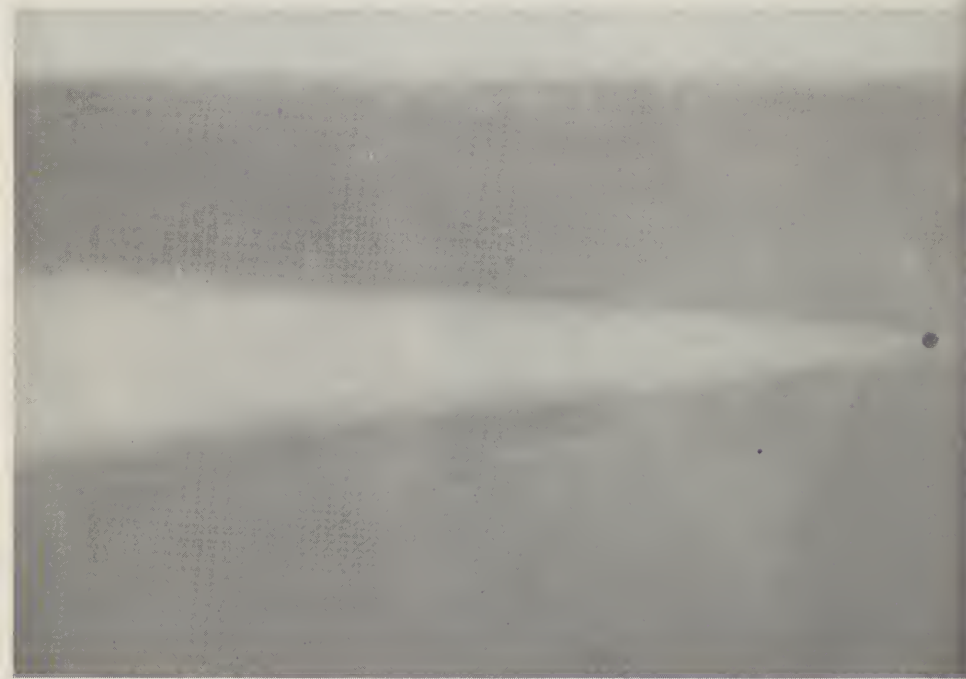
FIGURE 7. *Microsecond roentgenograms of a* $\frac{4}{32}$-*inch steel sphere passing through butcher's meat and through water.*

direct-current relay and interrupting the supply of alternating current to the manual control. The instrument is calibrated empirically, sensitivity being controlled by the size of the metering condenser and the voltage applied to the resistor network of the phototube.

The detector unit for general radiography (Figs. 4, 5, and 6) is similar to the detector of the exposure meter, except that its fluorescent screen is larger and a one-stage amplifier is provided to reverse the electrical sign of
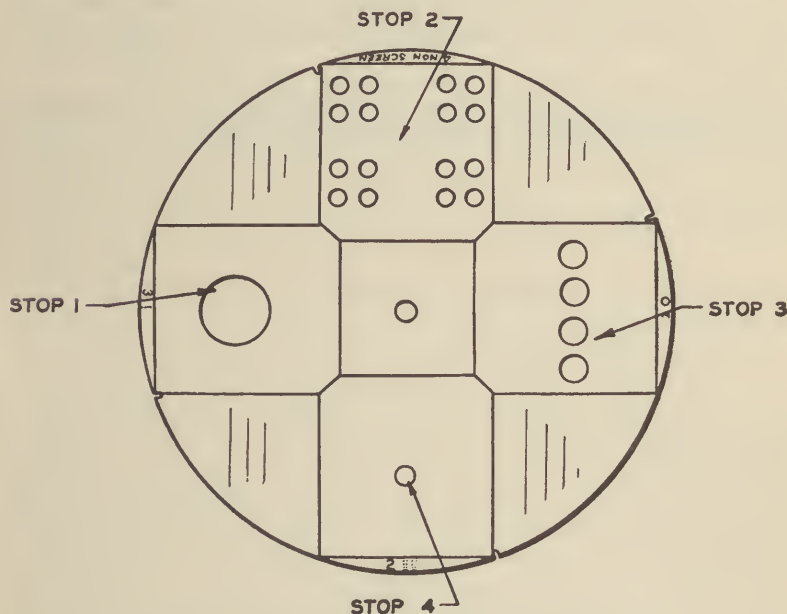
FIGURE 6. *Stops employed for table x-raying.*

the phototube current. The chassis includes a voltage-compensating circuit to improve parallelism between the response of film and of the detector over a considerable range of x-ray tube voltage. Interposed between the film and the detector is a lead-shielded disk, which mounts four apertures or stops. By means of suitable electrical connections to the chassis the sensitivity of the detector is adjusted to the size of the aperture being used.

SIGNIFICANCE OF WORK

The exposure meters have a permanent usefulness as laboratory instruments and their circuits are basic to the phototimer, but with the development of phototimers meters have become clinically obsolete. Phototimers

for photofluorography have made many hundreds of thousands of micro-films for the Army, Navy, and United States Public Health Service. Most of the commercial photofluorographs coming on the market in this country are provided with built-in phototimers.

Thus far the only phototimers used for general radiography are those at the University of Chicago, in the Army's Vaughan General Hospital at Hines, Illinois, in the Percy Jones Hospital at Battle Creek, Michigan, and in the Navy Medical Center at Bethesda, Maryland. There is every indication, however, that such phototimers will be generally available within a few years.[1]

[1] For further details and a bibliography, the reader is referred to the *American Journal of Roentgenology and Radium Therapy,* 43.474–482 (May, 1945).

STUDIES ON WOUND BALLISTICS

E. NEWTON HARVEY

THE SCIENCE of the motion of projectiles is conventionally divided into three parts — interior, exterior, and terminal ballistics. Interior ballistics deals with the behavior of missiles within the gun, exterior ballistics with their flight through the air, and terminal ballistics with the events after striking a target. Wound ballistics is a branch of terminal ballistics, having to do with the phenomena that occur when a missile strikes and penetrates the body.

Although a mass of information has been gathered on trajectories, stability, spin, yaw, and precession of projectiles in the air, rather less attention has been paid to the events that occur when a missile enters the body. This situation has been in part due to the rapidity of changes that take place in an opaque medium and the difficulty in measuring them, and in part to the complexity of the body and the belief that few significant generalizations can be made regarding it. Actually, the changes that occur when a high-velocity bullet penetrates soft tissue are remarkably independent of body structures and a common series of events can be outlined. The recent technical development of high-speed cameras that can take moving pictures at the rate of 8000 frames a second and x-ray apparatus that requires only $1/1,000,000$ second for exposure have eliminated the previous barriers to an understanding of the mechanism of wounding. It is now possible to analyze events that are completed in a few thousandths of a second.

Wound ballistics has two aspects. One is a determination of the factors involved in injury and the relation between the severity of the wound and such characteristics of the missile as its mass, density, velocity, shape, momentum, energy, and power. The attempt is made to relate the ability to wound or to kill with some physical property of the projectile. Such inquiry gives an answer to the question as to whether an anti-personnel bomb is more effective if it breaks into a large number of small fragments or a smaller number of relatively large fragments.

The second aspect of wound ballistics involves a study of the nature of the damage to tissues, whether this results from stretching and displacement or from pressure changes accompanying the shot. Of particular interest is the commonly observed injury of organs far away from the bullet path.

Such knowledge greatly aids the surgeon in his treatment of the wound and is necessary for the establishment of rules for removal of dead tissue and the amount of débridement necessary for proper recovery. The knowledge of wound ballistics is therefore important not only in offense but also in defense.

The scope of wound ballistics investigation sponsored by the Committee on Medical Research can be inferred from descriptions of the three projects dealing with this subject. The first, set up at the request of the Ballistics Research Laboratory of Aberdeen Proving Ground, was concerned with a "quantitative study of magnitude of wounds produced in tissues of various densities, elasticities, rigidities, and mixed structure, by fragments of different mass, velocity, shape, direction of hit, and angle of hit, together with the protective effect of clothing and armor." Time was insufficient to carry through the ambitious program outlined above, but quantitative data were obtained from which it is now possible to predict exactly what will happen when high-velocity steel spheres of small size enter soft tissue or bone — how much destruction of tissue occurs, how they are retarded, and how far they will penetrate. The behavior of fragments, as contrasted with spheres, has also been thoroughly investigated.

The second project had to do with investigations of reactions of the spinal cord to injury. The third or Survival Project was set up to determine the subsequent fate of animals with gunshot wounds of various degrees of severity and the best treatment for proper healing.

In all the above projects cats and dogs deeply anesthetized by nembutal have been used for study. The missiles were steel spheres $\frac{3}{32}$ to $\frac{1}{4}$ inch in diameter (weighing 55 mg. to 1041 gm.) or steel fragments of corresponding size. They were embedded in a split cylindrical holder or sabot and shot from a .30-caliber gun, using different amounts of a special powder. The velocities ranged between 1000 and 5000 ft./sec. By reducing proportionally the size of the animal and that of the ammunition, the effect on man of a standard Army 9.6-gm. bullet could be imitated.

The surgeon sees only the final wound and is frequently astonished by the extent of damage. With the unusually high velocities of bomb-burst fragments, a small splinter of steel can completely wreck a large volume of tissue. The manner in which this extreme damage takes place can best be described under the heading "Mechanism of Wounding."

MECHANISM OF WOUNDING

When a bullet moves through soft tissue such as muscle, viscera, or brain, one may expect to find a cylindrical core of completely pulped and disintegrated cells in its wake. The diameter of this elongated cylinder would presumably be at least that of the bullet and probably somewhat larger. The

missile may be thought of as plowing through tissue in the manner of a rod plunged into soft snow, which piles up in front of the rod and is pushed ahead; when the rod is withdrawn a hole is left whose diameter is little more than that of the rod. Such a process does occur in the body if the missile is one of relatively low velocity, such as a spear or a nearly spent revolver bullet. Damage to tissues is largely the result of their immediate displacement by the moving projectile.

When, however, the velocity of a missile is higher — the 2000 to 4000 feet per second of modern guns or the even higher speed of bomb fragments — an entirely new set of phenomena occurs. Immediately behind the bullet there is not a cylindrical hole but an ever widening cavity whose maximum diameter may be many times that of the missile. If the missile is a sphere and the tissue is homogeneous, the cavity will initially be cone-shaped, with the sphere at the apex, as shown in Figure 9. If the missile is a cylindrical bullet or a fragment, which tumbles, the cavity assumes extraordinary shapes (Fig. 20). The presence of bone or skin will also distort the cavity. Whatever the shape, the effect of the bullet's passage may be described as a little explosion in the tissue. Indeed, at the time high-velocity rifles were first perfected this action led to the mutual accusation by both sides in warfare that the enemy was using explosive bullets. Such an effect was recorded by Hugie in Paris as early as 1848.

The result of the large cavity formation is extensive damage to tissue at a considerable distance from the path of the bullet. This damage consists not only in destruction of cells of the tissue concerned but in rupture of small blood vessels, excessive stretching of nerves, and actual breaking of bone several centimeters away. Thus, the particular characteristic of high-velocity bullet wounds is disintegration of a large volume of tissue surrounding the bullet path.

The explosive action of a high-velocity missile is to be observed in any medium, even in steel, but is particularly characteristic of liquids, no matter how viscous. Many different media have been used to study the behavior of bullets. In wet mud or modeling clay the conical cavity is permanent. In dough or gelatin gel the cavity is mostly temporary but a smaller permanent cavity is left behind. In tissue also the cavity is mostly temporary but a smaller permanent cavity that fills with blood remains.

The soft parts of the human body contain about 80 per cent water. It is now quite certain that the behavior of a missile in any soft part of the body (excluding bone, cartilage, and tendon) is analogous to that in water. The law that determines the slowing of a missile in water, its retardation, also holds for soft tissues, although the numerical value of the constants may differ. Since water is a clear medium, it is admirably suited to photographic methods, and fundamental laws can be established with great ease and accuracy. The newer technics of high-speed motion pictures (2000 to 8000

frames per second) and spark photographs with exposures of 1/1,000,000 second reveal clearly the various phenomena in water or other transparent media, like gelatin gel.

The final proof of similarity of behavior in water or gelatin on the one hand and in soft tissues of the body on the other comes from microsecond roentgenograms. This remarkable development, perfected just before the war, allows an x-ray picture to be taken with a 1/1,000,000-second exposure. When a reliable trigger device is used, an x-ray picture of the bullet can be taken at any point in its path through opaque tissues and the accompanying changes recorded. In Figure 7 there is reproduced a microsecond roentgenogram of a sphere that has penetrated butcher's meat (above) and water (below). The similarity in appearance is obvious.

When a high-velocity steel sphere hits a surface of water, gel, or tissue, the momentary pressure at the front is enormous. The liquid is compressed and this region of compression moves out from the point of impact as a shock wave. Since the shock-wave velocity is that of sound in water (4800 ft./sec.), the wave rapidly progresses ahead of the steel sphere, now retarded by the water, as shown in Figure 8. Thus, the first event in penetration by a bullet is similar to that in an underwater explosion, where shock waves likewise form. Figure 9 shows the shock wave that arose from the detonation of a small cap, as well as the cavity formed about the explosive. The possible destructive effect of shock waves from impact of missiles will be considered shortly.

As the sphere progresses further and further, it expends its remaining energy in accelerating the medium, which is pushed sideways as well as forward, thereby leaving a conical cavity in its wake, also shown in Figures 7, 8, and 10. At a certain point in the path, the conical cavity separates from the sphere, constricts, and changes shape, repeating the expansion and contraction several times. The cavity finally resolves itself into a mass of small air bubbles that rise to the surface of the water. The sphere continues to move a short distance with a smaller cavity attached to it. The series of changes is shown in Figure 10, the first minimum volume in the pulsations of the cavity appearing in frame 25. It is significant that gas bubbles are frequently found in wounds from bomb fragments.

The formation of a large, initially cone-shaped cavity, which pulsates several times before subsiding, is also to be observed in 20 per cent gelatin gel, as illustrated in Figure 11, which reproduces a series of frames from a high-speed motion picture. These two series of photographs show a steel sphere and a rectangular steel fragment fired horizontally into gelatin confined in a tank with plexiglas sides. The expansion and contraction of the gelatin gel is apparent in the pulsating cavities that move back and forth along the old bullet path. Note that the cavity formed by the fragment is irregular. The permanent cavity left in the gel is also shown in Figure 11. If the

gelatin is in the form of a rectangular block resting on a glass plate, the glass may be broken even though the sphere passes an inch away from the plate.

The behavior in gelatin gel is exactly comparable to that which occurs in tissue. If a shot is fired through the thigh of an anesthetized cat or dog, high-speed motion pictures reveal a large momentary swelling under the skin, with subsequent pulsations, and a return to normal size in a few thousandths of a second. These changes are illustrated in Figure 12 from a high-speed motion picture of a shot through the leg of a cat, with the skin intact but the fur shaved to clarify the movements. Only part of the first pulsation is reproduced, the remaining pulsations occurring in later frames.

Superficially, not much damage to the leg is apparent, but on anatomical examination there is found a permanent cavity filled with blood and tissue débris in the missile path and a surrounding region where small blood vessels have broken and blood has extravasated into the tissue spaces. The two areas are readily seen as a cavity and a dark region in cross sections of the tissue normal to the path of the missile. Figure 13 is a photograph of a series of such sections cut on a meat slicer. Histologic examination reveals damage to muscle in a region near the permanent cavity but further out, breaking of capillaries only, and no visible damage to muscle fibers, which remain intact. A bone may be fractured at some distance from the path of the missile.

High-speed photography of a leg reveals only the external changes, and what goes on inside must be inferred from analogous shots into water or gelatin. That these inferences are correct and that the changes in a leg or abdomen are exactly similar to those in water can be established by a series of the microsecond roentgenograms referred to previously. Figure 14 shows successive stages in the expansion and subsidence of a cavity from a high-velocity sphere penetrating the thigh of an anesthetized dog. Figure 15 shows the maximum temporary cavity from a high-speed steel sphere shot through the thigh of a cat. The three prints are of the leg before (at left, an ordinary roentgenogram), during (in middle, a microsecond roentgenogram), and after the shot (at right, an ordinary roentgenogram). Note that the bone is broken although the sphere passed some distance from it. Figure 16 is a microsecond roentgenogram of the cavity from a fragment, and Figure 17 shows initial cavities from four balls that have penetrated the thighs of cats.

The same fundamental changes occur in the viscera as occur in muscle tissue. Entrance and exit holes in the skin are small, but internal damage is great. The chief differences are connected with the size and shape of the maximum cavity, which may be somewhat confined by skin or other anatomical structures. Figure 18 shows the cavity in the abdomen of a cat that has been fed a barium meal. Separate portions of the alimentary tract can be recognized, and their displacement by the temporary cavity will be noted.

In this roentgenogram the cavity is nearly maximum in volume. On autopsy it is not surprising to find segments of the intestine severed, if they are directly in the path of the sphere, but numerous small perforations occur well away from the missile track. These are believed to be the result of gas expansion due to pressure changes, which will be described shortly.

A high-speed moving picture (Fig. 19) of a shot through the abdomen of a cat reveals the great swelling due to the temporary cavity, followed by marked constriction. Wavelike movements of the abdomen then occur, followed by muscular contraction at a much later time, not shown in the motion-picture frame. On the other hand, a shot through the chest results in very little expansion or motion, owing to the fact that the thorax is primarily an air-filled region, so that not so marked a temporary cavity can form. In addition, the thorax is well supported by the ribs.

Previous mention has been made of irregular cavities in liquids. By shooting fragments rather than spheres into water and recording the results with high-speed photography the difference in behavior is easily seen. The energy delivered to the water depends, among other factors, on the projected cross-sectional area of the missile. Since the width of the temporary cavity in the water is determined by the energy delivered at that particular penetration distance, the shape of the cavity is an index of the orientation of the fragment. Whenever the fragment turns broadside much energy is delivered and a large cavity forms, and whenever it turns end-on less energy is absorbed and a small cavity results. However, resistance to the passage of the fragment is less with end-on orientation and the penetration is greater. Figure 20 shows some of the bizarre cavities formed by fragments shot into water. From such studies it can be concluded that to inflict serious damage a missile should hit head-on, but should turn broadside when it penetrates near a vital organ, with the delivery of a large amount of energy in a short distance and consequent formation of a broad cavity.

The head of an animal presents a special problem. Here the soft brain is confined in a strong and rigid cranium. The expansion of the cavity is restrained by the skull, and a very high pressure may be suddenly built up within — a pressure great enough to burst the bones apart. The cavity formation from a shot through the skull of a dog is shown in the microsecond roentgenogram of Figure 21. In no region is the explosive action of a high-velocity missile more apparent than in the cranium. It is well known that large regions of the brain of man may be reduced to pulp by a high-velocity shot through the head and that the head bones are literally blown apart, frequently along suture lines. On the other hand, a low-velocity shot may penetrate and cause only local damage.

Many of the observations on the battlefield can be imitated by shooting ⅛-inch steel spheres of different velocities into the intact head of an anesthetized cat. A low-velocity sphere (1100 ft./sec.) leaves neat entrance and

exit holes in the skull, with no splitting of sutures or fracturing. Spheres of somewhat higher velocity (2200 ft./sec.) show entrance holes with jagged edges or slight fractures and occasional splitting along a suture line. A 3000-ft./sec. sphere causes considerable suture splitting and fracturing, and a 4500-ft./sec. sphere results in complete shattering. The skull of a cat whose head has been penetrated by a 3800-ft./sec. sphere is shown in Figure 22 (below). The bones were completely broken apart.

On the other hand, if the brain is removed from the fresh head of a cat through the foramen magnum, leaving the skull full of air, and the latter is shot with a ⅛-inch steel sphere moving at 3800 ft./sec., there is no shattering or fracturing of skull bones. Such a skull is shown in Figure 22 (above). The entrance hole is larger (about ⅜ inch across) than the exit hole, which is a neat circle, the exact size of the steel sphere. Without a liquid interior no temporary cavity will be produced, and hence no pressure can develop to blow the skull bones apart.

Likening the effect of a high-velocity bullet to a small explosion is by no means far-fetched. The comparison is so exact that it is possible to say how many milligrams of TNT exploded within the body will produce a temporary cavity whose maximum volume is the same as that of a missile of given weight striking the body with a given velocity. Moreover, the explosion of large amounts of TNT under water gives rise not only to a pulsating cavity but also to shock waves whose peak pressures are measured in tons per square inch. These shock waves from detonations can kill a person near enough to the explosion and may break tissues whenever they reach a free surface; for example, a surface between air and liquid, as in the lung, or at gas pockets in the intestine. Hemorrhage in the lung and perforation of the intestine may result.

The possibility that shock waves, which arise when a missile hits the body, may damage tissue is very real. Each wave is made up of a sudden rise of pressure followed by a rapid and then a slower fall of pressure, which may go below the atmospheric level before returning to normal. Peak pressures of the order of 800 lb./in.^{2} have been observed from ³⁄₁₆-inch spheres striking water with high velocity. The measurement is made at a distance from the point of impact greater than the maximum extension of the temporary cavity. In water the pressure falls off directly with the distance, but in tissue the shock waves suffer reflection and scattering and the intensity diminishes rapidly. A negligible movement of water accompanies the wave.

The fundamental question to be answered is whether the pressure in the shock wave has fallen to some harmless low value at a point beyond the greatest expansion of the temporary cavity; otherwise it would be difficult to separate shock-wave destruction from expanding-cavity destruction. Experiments indicate that shock waves from missiles, which produce profound destruction of a large volume of tissue, are not important in wound-

ing, provided there is no air in the tissues. This statement applies to the masses (0.05 to 1.0 gm.) and velocities (2000 to 4000 ft./sec.) used in the Princeton experiments. The evidence comes from the behavior of frog hearts (free of air) tied in position in Ringer's solution at different distances from the path of a high-velocity sphere, shot into the solution. The hearts that were rapidly moved by the expanding cavity (even though they were not engulfed by it) were injured, as shown by a slowing or cessation of heartbeat. These injured hearts, in addition to displacement by the temporary cavity, were also struck by 600-lb./in.^{2} shock waves. However, in other experiments where frog hearts were tied at a distance from and not rapidly moved by the expanding cavity, they were found to be uninjured, even though they were struck by shock waves of much greater intensity. These experiments on gas-free tissues, together with others also indicating that shock-wave effects from bullets are not very violent, have led us to the conclusion that it is the rapid displacement and stretching of tissue by the expanding cavity in the wake of the missile, rather than the shock wave, that is the important factor in wounding.

In addition to pressure changes in the shock wave there are also pressure changes accompanying the movements of the cavity. During the initial expansion of the cavity, pressure in the water nearby is greatly increased. As the cavity expands further, there is a change to a greatly decreased water pressure, perhaps that of water vapor, the same pressure as exists in the cavity itself. The origin of this subatmospheric pressure lies partly in the Bernouilli effect of the rapidly moving water and partly in the inertia of the water system. The water, pushed sideways by the missile, keeps moving outward and develops tension, causing the cavity to overexpand. With later contraction of the cavity the pressure again increases and the cycle is repeated, as indicated by pulsation of the system.

The magnitude of these pressure changes around the cavity is not as great as the peak pressures of shock waves, but the cavity pressures last a longer time. Many experiments indicate that cells can stand high hydrostatic pressure provided no gas is present. It is not the pressure changes around the cavity that do harm but the great displacement, stretching, and tearing of tissue.

Whenever gas is present, such as air in the lung or gas pockets in the intestine, this gas may expand, provided it is near enough to the path of a high-velocity missile. The gas-pocket expansion is due to the low-pressure phase of cavity expansion described above. The rapid displacement of tissue by the expanding gas pockets may result in serious damage. In the experiments with frog hearts used to test the lethal action of shock waves, it was found that whenever air was injected into the ventricles the hearts were invariably injured. The actual expansion of the injected air could be observed in motion pictures of the hearts near the temporary cavity. Perforation of

the intestines at a distance from the path of a shot through the abdomen, mentioned previously, may also be explained by the expansion of intestinal gas in the low-pressure region around the temporary cavity. The injury to tissues near gas pockets away from the path of a bullet through the body may be spoken of as secondary damage.

Any tissue gouged out by the moving projectile, which actually excavates a permanent cavity whose cross-sectional area is somewhat greater than that of the missile, must be completely destroyed. In addition, a region represented by the area of extravasated blood is very seriously damaged, and finally, the region included in the area of temporary cavity is severely stretched. A tissue sufficiently elastic may stand the stretching without a break. In one instance an intact artery was observed spanning the permanent cavity; in another blood vessels running with the nerve in the same fascia have been unharmed although the nerve was broken.

The problem of peripheral nerve injury is undoubtedly bound up with the stretching of nerve during the expansion of the temporary cavity. A direct central hit on a nerve may occur, but even if the nerve is not squarely hit, but merely pushed aside by the tip of the bullet, it will be carried along with other tissue by the expanding cavity and stretched to the point of breaking. It is the nerves that are stretched without breaking that present the most interesting situation, for many cases of paralysis from peripheral nerve injury have been recorded where no visible nerve damage was apparent.

A special study of the stretching of sciatic nerve by high-velocity missiles has been made by the Princeton group. Cats anesthetized with nembutal were wounded with ⅛-inch steel spheres moving at velocities of 3200 to 4100 ft./sec. The sphere passed through the fleshy portion of the thigh in the lateromedial direction or vice versa, perforating the biceps femoralis, adductor femoralis gracilis, and semimembranosus muscles. After the shot the nerve was exposed at the level of the sciatic notch and tested for irritability by stimulation, the contraction of various leg muscles serving as indices of response. If no muscle contraction occurred on stimulation, the nerve was exposed along the thigh, carefully examined for injury, and tested for irritability at various levels.

It was found that in order to affect nerve conduction it was necessary for the sphere to pass within 1.0 to 1.5 cm. of the sciatic nerve, depending on its velocity and mass. In 22 experiments, 15 cases showed no visible injury or impairment of conduction. The missile path was several centimeters from the nerve. In 3 cases the nerve was severed in whole or in part, and conduction was lost. In the remaining 4 cases, no visible external injury to the nerve was apparent, although conduction had ceased; 2 of these cases were followed for six hours and no recovery occurred in that time. The paralysis appeared permanent, at least until regeneration could take place.

The stretch of a nerve by the expanding cavity can be seen in microsecond roentgenograms. It is best to record this phenomenon in a plane giving a cross section of the expanding cavity and the wound. This means that the x-ray beam must parallel the path of the missile, which penetrates the tissue as well as the casette with its film and intensifying screens. A positive print of such a roentgenogram is reproduced in Figure 23. The temporary cavity is circular in outline and the nerve, which has previously been injected with iodobenzene to render it radiopaque, is stretched into a loop to the left of the cavity.

Histologic studies of the 4 sciatic nerves that showed no visible damage externally yet lost their ability to conduct nerve impulses revealed internal injury. In all the cases some fibers were broken, and many were considerably separated from each other and presented a wavy appearance. Sometimes the axis cylinder was broken while the medullary sheath and neurilemma were intact. The nerve sheath (epineurium) was always intact. Loss of conductivity with consequent paralysis must be associated with the broken or injured fibers.

The stretching of blood vessels by a high-velocity missile can also be readily demonstrated by x-ray technic. Figure 24 is a print of a microsecond roentgenogram taken in the same manner as the previous one with nerve. It shows a blood vessel injected with barium sulfate (basolac), which has been stretched and bowed by the expanding cavity. An ordinary x-ray photograph taken after the shot proved that the blood vessel was not broken but returned to its original position with the subsidence of the temporary cavity. Microsecond roentgenograms thus supply unequivocal evidence of the stresses to which tissues in the body are subjected during the passage of a high-velocity missile.

The experimental findings in the field of wound ballistics may be summarized by a list of the important events and principles in wounding by high-velocity missiles, as follows:

(1) The generation of shock waves at the point where the missile strikes the skin. These shock waves are pulses of high pressure, which pass through the tissues with the speed of sound in water. Little displacement of tissue accompanies them.

(2) The development in the wake of the missile of a temporary cone-shaped cavity partially filled with air and water vapor, many times the cross-sectional area of the missile and involving great displacement of tissue.

(3) Pulsation of the cavity. After reaching a maximum volume in some tissues, the cavity decreases in size and then increases again, repeating the volume changes several times before subsiding.

(4) The formation of a permanent cavity filled with blood and tissue débris, whose cross-sectional area is somewhat larger than that of the missile.

(5) As a result of the development of the temporary cavity, tissue is dis-

placed, blood vessels and nerves are greatly stretched, and bone may be broken.

(6) Where the tissue is displaced sufficiently, capillaries and small blood vessels may rupture, leading to a well-defined region of extravasated blood, smaller than the temporary cavity but larger than the permanent cavity.

(7) It can be demonstrated experimentally that neither the slight displacement nor the pressure change in a shock wave is sufficient to damage tissue provided no gas is present.

(8) In addition to great displacement of tissue that results from the expanding temporary cavity, there are also pressure changes associated with it. In the initial stages of expansion tissue surrounding the cavity is under increased pressure, but this increase is quickly followed by a decreased pressure due to Bernouilli effects and to overexpansion of the cavity. In the absence of gas in the tissues, it is the displacement, accompanied by stretching and tearing, that is of primary importance in damage to tissue. If gas is present near a temporary cavity, the expansion of gas during the decreased-pressure phase of overexpansion may itself tear and further injure tissue, giving rise to secondary damage.

As a consequence of the above events and principles, many of the more specific observations in connection with high-velocity wounds find a rational explanation. Some of them are:

(1) Hemorrhagic consolidation of the lung by a shot near to but not hitting it, a consequence of the expansion of the alveolar air, due to the decreased pressure from overexpansion of the temporary cavity.

(2) Perforation of the intestine by a shot not hitting the intestine, due to expansion of intestinal gas by the mechanism mentioned above.

(3) Splitting of muscles along fascial planes, resulting from cavity expansion along lines of least resistance, often leaving a scalloped wound.

(4) Breaking of a long bone by a near miss, resulting from the pressure of moving tissue on the bone.

(5) Blowing apart of skull bones along suture lines, due to the increased pressure of the expanding cavity within the skull.

(6) Loss of nerve function without external injury, from excessive stretching of fibers.

(7) Immunity of large blood vessels present in a wound where small vessels may be broken. The large vessels are elastic enough to stretch, while most other tissue is destroyed.

(8) Disintegration of a large mass of tissue under the skin, despite a small entrance and exit hole in the skin itself. The profound disintegration of inner tissue is connected with the temporary cavity expansion; the small skin holes are due to the greater structural resistance of skin to expansion and to its elasticity, allowing it to return to nearly the original size, despite a temporary stretch.

REACTIONS OF THE SPINAL CORD TO INJURY

Previous studies by Windle and his associates on injury to the spinal cord involved such procedures as exposure of the cord for up to sixty minutes by aseptic operation under varied conditions, as well as concussion from a blow in different regions. Both the operation and the blow resulted in functional disturbances, both motor and sensory. For example, a blow applied over approximately the sixth lumbar spinal-cord segment produced concussion, the severity of which was measured by the magnitude of increase and the duration of recovery time of the threshold for a femorosciatic reflex. The severity of the concussion and the longitudinal extent of spinal cord involved increased with the strength of the blow. A blow producing concussion at the seventh and eighth thoracic segments did not spread sufficiently to produce concussion of the region of the cord involved in the femorosciatic reflex, but blows spaced from the eleventh thoracic down to the third lumbar segments caused progressively greater increase in threshold for this reflex. However, a blow producing concussion at the seventh and eighth thoracic segments, as well as a blow at the lumbar enlargement, caused a rise in threshold of a femorofacial reflex.

If stimulation was applied in the pyramid to evoke hind-limb movement and slight forelimb movement, a blow at the lumbar enlargement or at the seventh thoracic segment caused a rise of threshold for hind-limb movement. Forelimb movement was little affected, although motor nerves for these movements originate from cord segments closer to the site of the blow.

In one fourth of the cases in which concussion was produced at the level of the lumbar enlargement, the hind limbs immediately extended in tonic spasm lasting for five to twenty-five seconds. The occurrence of spasm was unrelated to severity of concussion.

Uncomplicated concussion of the spinal cord in the cat, then, is a complete functional block of the spinal cord at the level of application of an adequate force to the nervous parenchyma. Nerve cells, principally interneurons, and long ascending and descending fiber tracts are involved. The period of concussion is brief, passing into the period of post-concussion when the paralysis ceases. The much greater depth of spinal shock to which man is subject perhaps adds to the profoundness of the functional alterations. Subtle but histologically demonstrable cell alterations occur in concussion, and chromatolysis is evident in post-concussion as a direct result of the physical injury of the cells sustained at the time of concussion.

It is well known that gunshot wounds of the vertebrae may produce cord lesions without damaging the neural canal. In order to compare the effects of gunshot with concussion from a blow, the Northwestern University group wounded 35 etherized cats in the region of the spinous processes of thoracic (25) and lumbar (10) vertebrae by ⅛-inch steel balls having impact velocities between 3000 and 3500 ft./sec. The severity of neurologic signs was
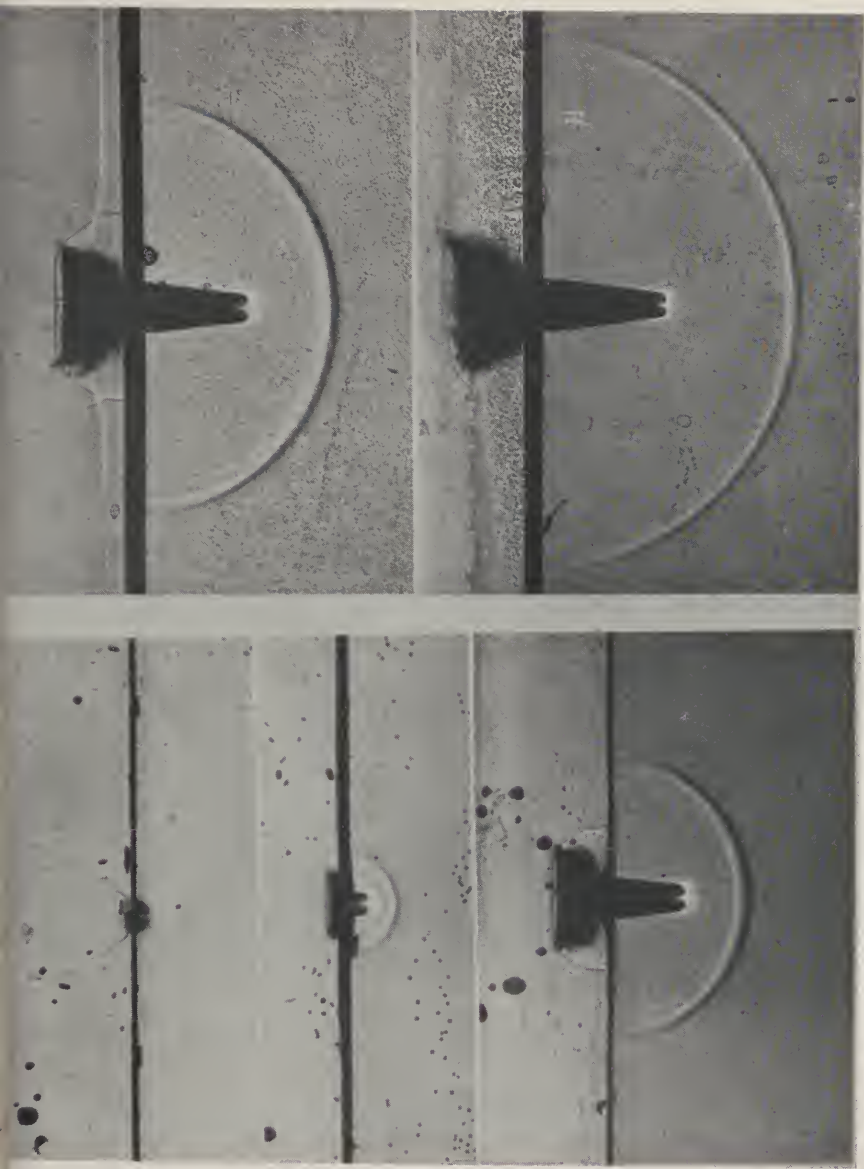
FIGURE 8. Spark shadowgrams of a 4/32-inch sphere taken at successively longer intervals after it has hit the surface of water.
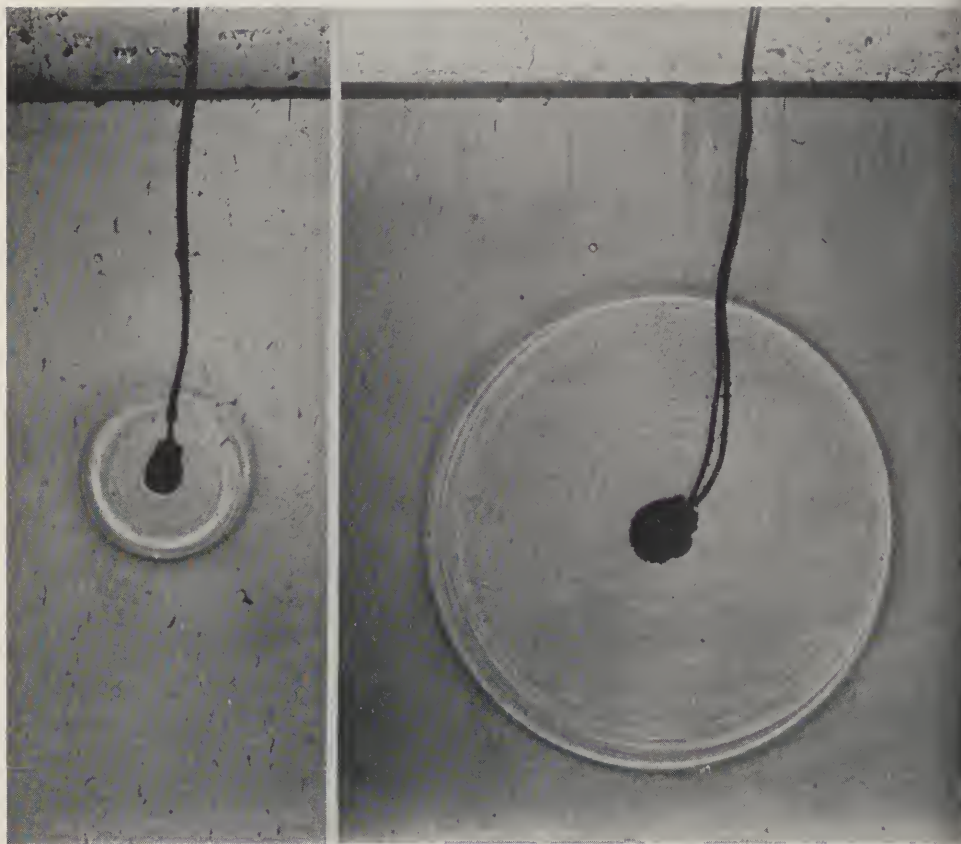
FIGURE 9. *Shock waves in water formed by the explosion of a small detonating cap.*
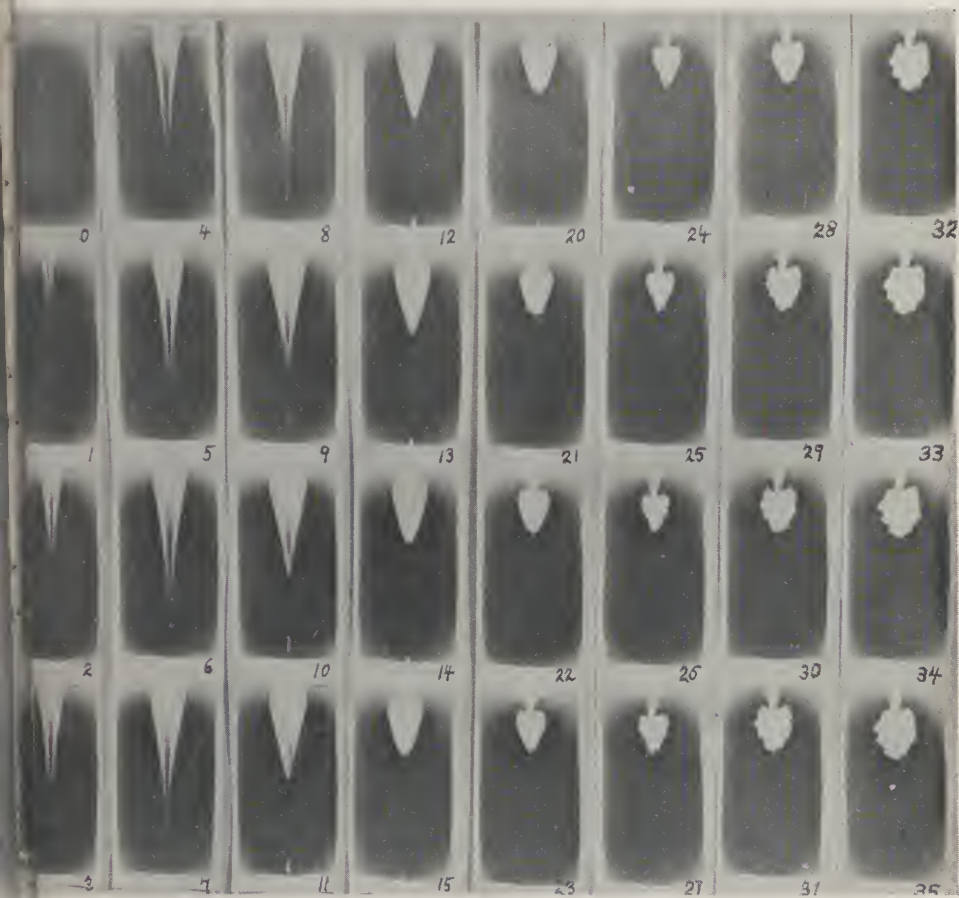
FIGURE 10. Negative prints of frames (1920 per second) from a high-speed motion picture, showing a $\frac{3}{16}$-inch steel sphere entering water with a velocity of 3160 ft./sec.
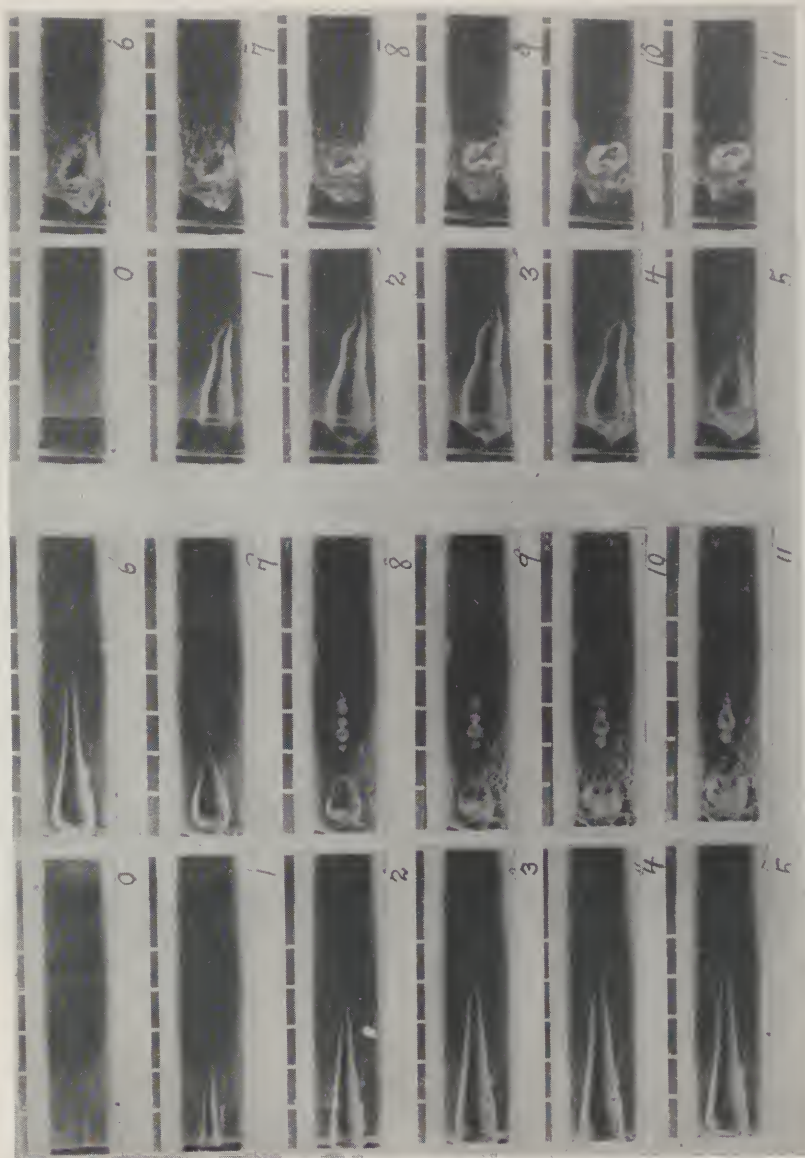
FIGURE 11. *Negative prints from a high-speed motion picture showing the effects of horizontal shots fired into 20 per cent gelatin gel, enclosed in a narrow tank with plexiglas sides.*

FIGURE 12. *A series of prints from a high-speed motion picture (4500 frames per second) of the leg of an anesthetized cat with skin intact, shot from right to left through the thigh with a $\frac{4}{32}$-inch steel sphere moving at 3000 ft./sec.*
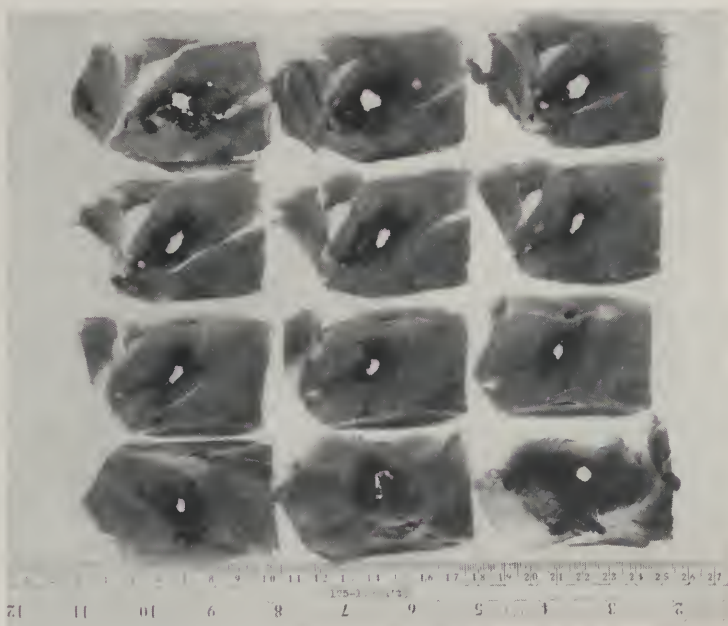
FIGURE 13. *Thick serial sections of the soft tissues of a cat's thigh, showing the effects of a missile.*
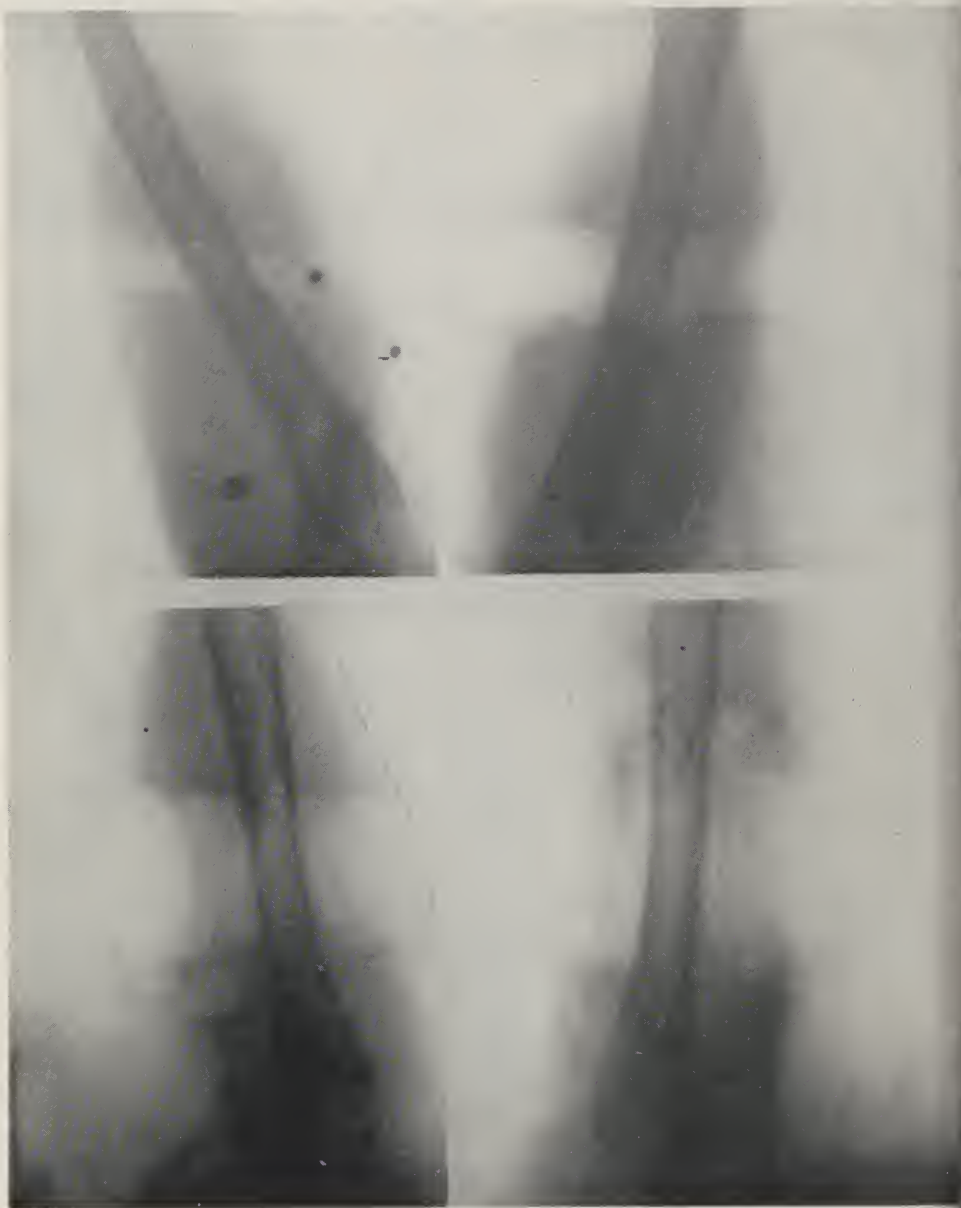
FIGURE 14. *Four microsecond roentgenograms of ⁴⁄₃₂-inch steel spheres, taken at successively longer intervals after a shot through a dog's thigh.*
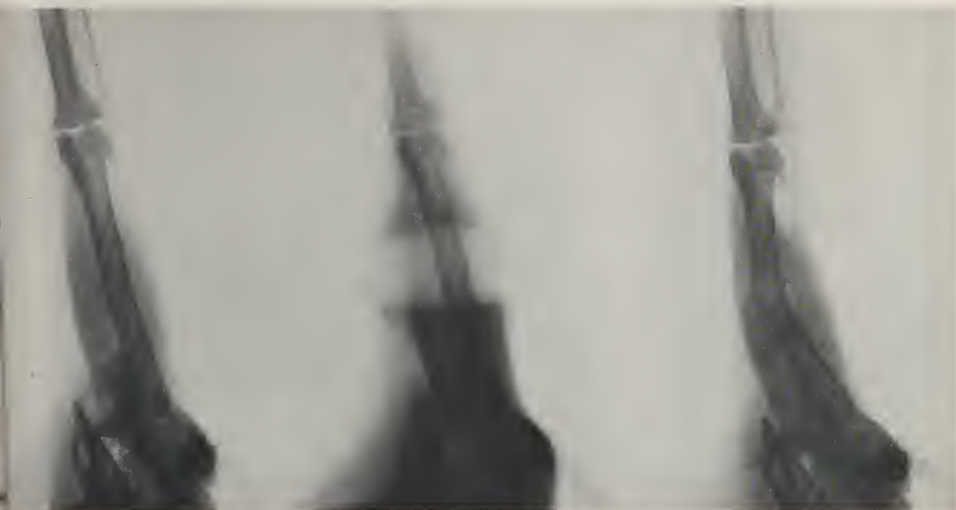
FIGURE 15. *Roentgenograms of a cat's leg before, during, and after the passage of a* $\frac{4}{32}$*-inch steel sphere with a striking velocity of 3000 ft./sec.*

FIGURE 16. *Microsecond roentgenogram of a cat's thigh, showing the effect of the passage of a section of a wire nail.*

FIGURE 18. Microsecond roentgenogram of a temporary cavity in a cat's abdomen, caused by the passage of a $4/32$-inch steel sphere.

FIGURE 17. Microsecond roentgenogram of a cat's thigh showing early stages in the formation of a cavity caused by four $4/32$-inch steel spheres.
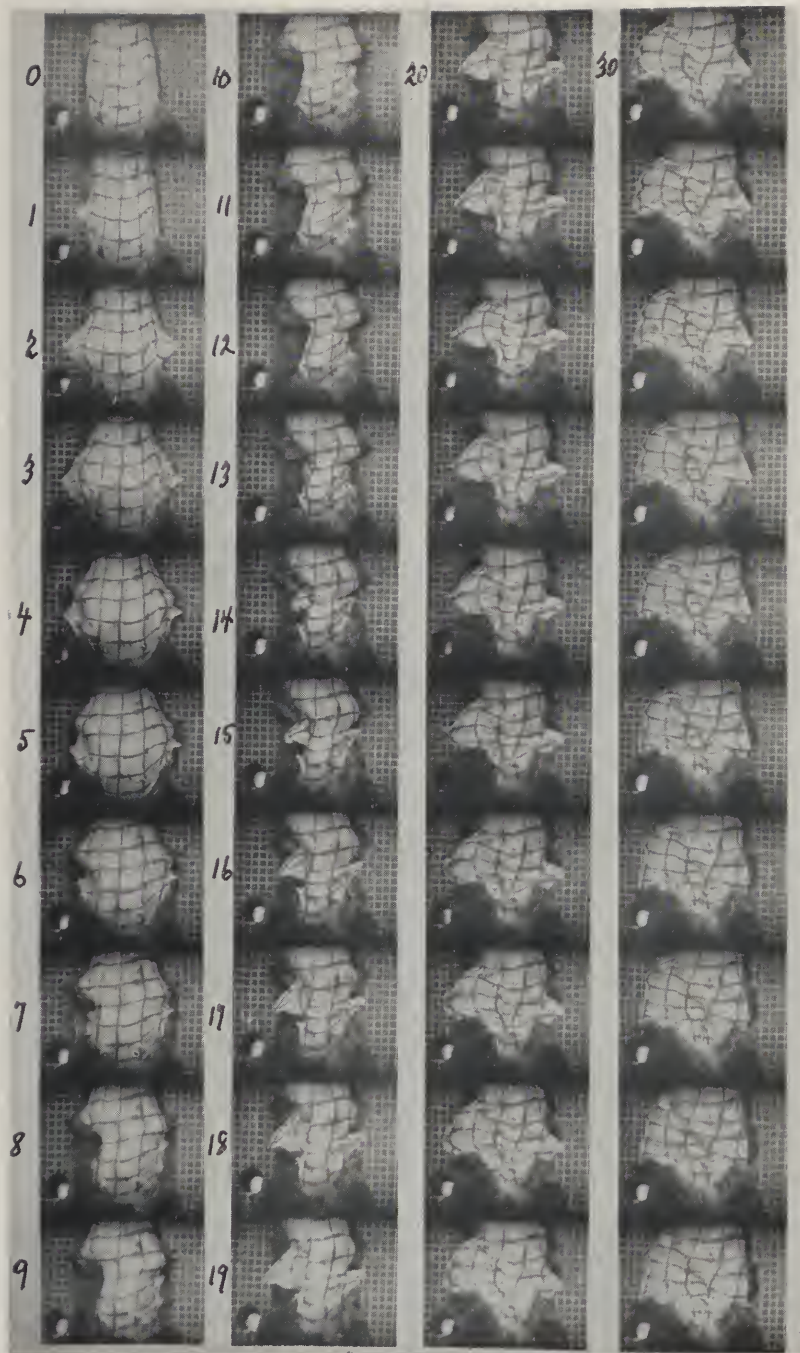
FIGURE 19. Frames (2880 per second) from a high-speed motion picture of a cat's abdomen showing the volume changes and movements caused by a $^6\!/_{32}$-inch steel sphere.

Figure 20. *Irregular temporary cavities formed in water by fragments rotating during penetration.*

Figure 21. *Microsecond roentgenogram of the head of an anesthetized dog, showing the effect of an ⁸⁄₃₂-inch steel sphere.*
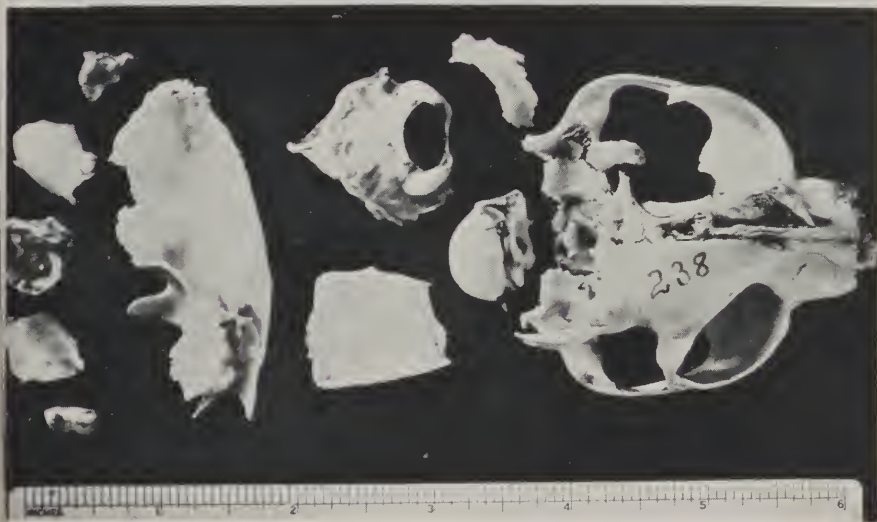
FIGURE 22. *Relative effect of a missile on the skull of a cat, with and without the brain removed before the shot.*
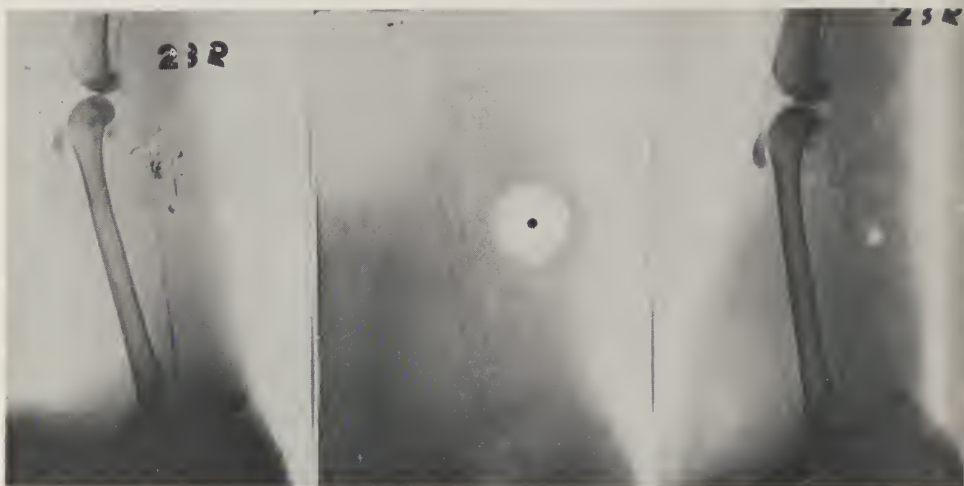
Figure 23. *Roentgenograms of a cat's thigh taken immediately before and after shooting with a 1/32-inch steel sphere. (See Fig. 15.)*
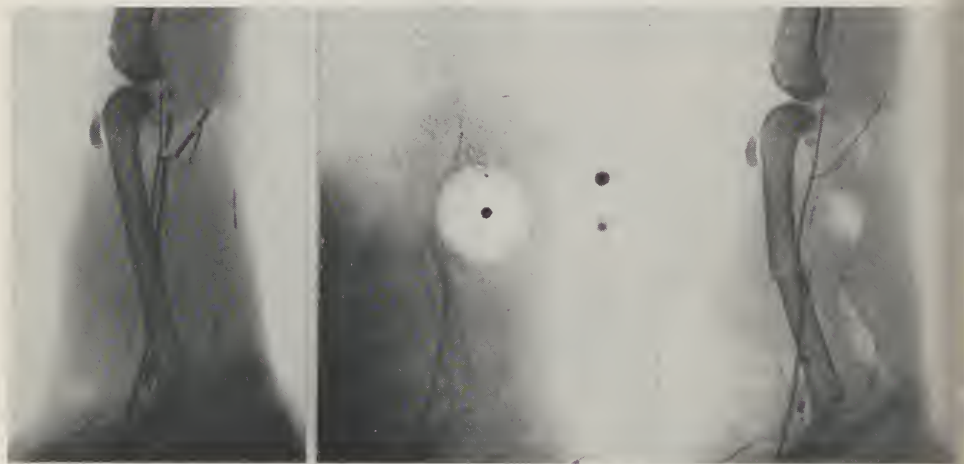
Figure 24. *Roentgenograms of a cat's thigh (same as Fig. 23) showing displacement of blood vessels.*

then correlated with the location of the wound. In all cases the neural arch was undamaged.

It was observed that shots passing the cephalic or caudal edge of thoracic spinous processes produced signs of severe cord damage, whereas shots striking the middle of thoracic spines produced minimal or no cord signs. The results suggest that rotation of vertebrae about a longitudinal axis is innocuous, whereas their rotation about a transverse axis causes damaging shear strain in the spinal cord. Bone damage does not always appear to accompany cord lesions in the thoracic region.

In the cat the mobility of lumbar vertebrae is limited. Severity of cord damage in this region varies with proximity of the shot to the thin roof of the neural canal. It appears that either the shock wave or momentary depression of the roof of the neural canal by the expanding cavity behind the missile produces compression of the spinal cord. Bone damage always accompanies lumbar-cord damage. It is therefore suggested that vulnerability of the cord to damage from hits or near-hits on vertebrae is determined in part by mobility of vertebrae in various portions of the vertebral column.

Histologic examination of the spinal cord after aseptic surgical exposure, after concussion, or after shooting a spinous process of a vertebra showed secondary pathologic reactions. In its mildest form the reaction consisted of a perivascular lymphocytic infiltration and pavementing of the same cells in small veins, appearing as early as three days after the injury. The reaction usually proceeded further, with graded pathologic effects in the form of more intense infiltration, glial proliferation, transformation of lymphocytes into gitter cells, fiber degeneration, chromatolysis, softening, and cavitation. The damage to nervous tissue seen at necropsy was predominantly secondary, since it was many times greater than the possible primary damage to perikarya or fibers occurring as a direct result of the disturbance to which the cord had been subjected.

SURVIVAL OF ANIMALS AFTER GUNSHOT WOUNDS

For this study the Yale University group produced standard wounds in the thighs of dogs under nembutal anesthesia and observed the subsequent recovering and healing. A $7/32$-inch steel sphere with a velocity of 5000 ft./sec. leaves a widely open wound with much loss of tissue, but the continuity of the femoral artery is preserved. Immediately after injury moderate bleeding occurs; the wound of exit is somewhat larger than that of entrance. There is gross evidence of dissection along the fascial planes at right angles to the sinus tract, and the tissues forming the walls of the tract are normal.

When such a wound is left untreated and the animal is allowed freedom in its cage, healing is relatively rapid and essentially complete in about three weeks. During the first few days after wounding the thigh is moderately swollen and ecchymotic. The sinus tract remains wide open throughout its

extent. A thin but relatively copious serosanguinous fluid exudes from the wound, but the walls of the sinus tract and the edges of the skin are free of conspicuous slough and are covered in part with a thin layer of fibrin. Granulation tissue promptly appears in the central portion of the wound, being clearly apparent within five days after wounding. The animal uses the wounded extremity for support within a few days after being wounded, and for progression within a week.

Closure of the large cavity is relatively slow for the first ten days. The skin wounds of entrance and exit close mainly by contracture, with some marginal ingrowth of epithelium. About three weeks after injury the surface wounds are either closed or present only a minute opening without drainage; the deeper sinus tract is either obliterated or represented only by a thin slit. Thereafter the site of the sinus tract is difficult to localize by dissection, since there is little scarring along its previous course. Spheres of smaller size and lower velocity produce correspondingly smaller wounds.

Several pertinent points should be noted in connection with this study. (1) This type of wound is wide open throughout its extent from the very beginning, and drainage is consequently excellent. (2) Freedom of movement is permitted the animal during the course of healing, and even at an early date active motion in the wounded extremity is frequent. (3) The animal licks the surface aspects of the wound but not the deeper portion. (4) The continuity of the femoral artery is preserved in the wounded extremity. (5) The wound is free of impacted foreign material. (6) The general behavior of the wound throughout the course of healing is similar to that of a traumatized soft-tissue wound without slough or impacted foreign material but provided with adequate drainage.

Wounds produced in a similar manner with a .22-caliber bullet heal with the same characteristics. The original wound is smaller, however, and heals more rapidly. When this latter type of wound is treated, within one hour after injury, by excision of the edges of the skin wounds and immediate closure of the skin edges with silk sutures (clean but not aseptic technic), the external wounds heal *per primam* and remain closed.

A rather unexpected aspect of the study was the absence of dead tissue in the wound. The wounded animals were not treated differently from intact animals, except that profuse hemorrhage was controlled by packing for a short period, and also, in a few cases, the wound was closed with clean but not sterile instruments. In a few cases, wounds have included very small tags of tissue on a thin stem, which would ordinarily, in surgical practice, be removed without applying the term "débridement." However, in no instance, at any interval after its formation, has a wound on gross examination contained a significant amount of devitalized tissue.

Since débridement is a well-recognized principle of surgery in military practice, the healing of these wounds without this procedure is of prime

interest. Several explanations may be advanced. First, there is the species difference: the dog may behave in a very different way from a man. Second, the site of the wound studied was restricted to the hamstring portion of the thigh; possibly other wounds might be more subject to devitalization. Third, none of the missiles passed through clothing. Fourth, the treatment of the dog differs from that of a man in that the dog was not immobilized nor were its wounds dressed; the early physiotherapy provided by the dog in moving about his cage and licking the wound may be important in healing. Finally, the possibility must also be considered that human wounds are in less need of débridement than has previously been thought, resembling the wounds seen in the untreated dogs described above.

The experiments on survival had led to a test for peripheral nerve interruption and degeneration that has been applied successfully to the experimental animals and also to human cases. This test is based on changes in direct-current surface potential differences following nerve block. It can be applied in cases of experimental gunshot wounds. The test makes it possible to determine whether or not peripheral nerves are severely injured as a result of the passage of a bullet through the limb. Such a method should prove of great value in diagnosis and should be given extensive trials.

THE FUTURE OF WOUND BALLISTICS STUDY

In any plan for continuation of the study of wounds the following four lines of approach should be kept in mind.

(1) Extension of the quantitative data already obtained by the Princeton group to larger (heavier) spheres and fragments, using much larger animals than the cat. Anesthetized sheep or goats are suggested. The following constants should be determined: drag coefficients in muscle and viscera; volume and shape of temporary cavity, extravasated blood region, and permanent cavity in muscle and viscera — all as a function of mass, density, projected area, and velocity of missile. Retardation of missiles by different kinds of bone should also be further studied.

(2) Simultaneous records of many physiological processes (heartbeat, respiration, blood pressure, brain waves, reflex movements, and so forth) when an animal is hit in various regions. The records should be continued for a long time after the hit, in order to evaluate physiological shock.

(3) Survival studies of the above animals to determine the subsequent history of tissues at different distances from the missile path, rate of healing after varying degrees of débridement, and possibility of infection.

(4) Special problems, such as the pressure distribution around the missile path, pressures and displacements within the skull, the pressure necessary to break bone, and the stretch of nerves and blood vessels near the missile path.

Part Three: Aviation Medicine

CHAPTER XIX

INTRODUCTION

DETLEV W. BRONK

I T IS the purpose of military aviation to increase man's power of attack and defense by giving him freedom of movement in three dimensions, the ability to travel at high speed with great maneuverability, and the power to climb far into the sky. Aerial machines thus have a biologic function. Accordingly, the progress of aviation has depended on the combined efforts of physiologists, physicists, and engineers.

Scientific progress brings new dangers with new human powers, and reveals new physiological limitations as old ones are overcome. The history of military aviation is replete with instances of this. Time and again physicists and engineers have developed machines that the flier has been unable to use; or the machines endangered the lives of those who used them. Then the progress of aviation again waited on the assistance of the biologist. Thus we have attained our present prowess in the air through the combined efforts of the physical scientist and the physiologist.

If this is not apparent to most people, it is because they have done their flying far behind the front lines of man's combat with the unknown forces involved in flight. Scientists and manufacturers and test pilots have faced the hazards and have overcome them before each new development has been given to the public. In military aviation this is not so. The demands of national security impose on military forces the responsibility for quickly adapting each new scientific discovery to their tactical and strategic advantage. In order to gain the advantage of time and surprise they have found it necessary to employ to the very limit each new advance in aviation.

This was especially true during World War II. The co-ordinated efforts of vast numbers of scientists, engineers, and manufacturers gave us with bewildering rapidity new planes, instruments, and weapons that would have matured but slowly in peacetime. Machines that severely taxed the human organism had to be operated under difficult and dangerous conditions in

order to protect our armed forces and the Nation. To make aerial operations possible under those difficult conditions and to protect the lives and efficiency of airmen was the mission of flight surgeons, aviation physiologists, and psychologists, and of biologists and medical scientists working in laboratories throughout the country.

Looking back to 1940, we find that the role of the civilian scientists in this undertaking was especially difficult. Few of them had any direct familiarity with the problems of military aviation; most of them had not flown in Army or naval aircraft; none had participated in modern military maneuvers. And yet the physiological dangers and stresses of aerial combat could only be recognized and comprehended by those who had experienced these problems. Nor were there more than a handful of regular officers in the Army and Navy who had both the practical knowledge of air force operations and the scientific training necessary to define for the civilian scientists the physiological difficulties that soon imperiled the success of our military campaigns.

Anticipating the potential dangers of this situation, Dr. Lewis H. Weed, Chairman of the Division of Medical Sciences of the National Research Council, organized a Committee on Aviation Medicine in the fall of 1940 with Dr. Eugene F. DuBois of Cornell Medical College as Chairman. This committee of physiologists, psychologists, and physicians immediately proceeded to educate themselves in these matters. They toured airfields where young men were receiving a belated training for the aerial war that soon came. They visited aircraft factories and discussed with aircraft designers the characteristics of the new planes that were to overstress the powers of the human body. They held frequent conferences with the leaders of the air forces who were planning the strategy and tactics of the campaigns to be fought far above the earth by young men accustomed to life on the ground. Fortunately, they had a valuable guide in Armstrong's admirable treatise, *Aviation Medicine,* which had recently been published. Unfortunately, there was only limited financial assistance for their study of these problems, made available by the Health and Medical Committee of the Council of National Defense through the National Research Council.

When the Committee on Medical Research of the Office of Scientific Research and Development was created in July, 1941, there was available a small group of scientists who had already formulated some of the more important physiological problems of human flight, owing to the previous activities of the Committee on Aviation Medicine. Accordingly, the Committee on Medical Research sought their advice in the organization of a great, co-ordinated program of research in aviation medicine.

The story of that undertaking is a remarkable account of co-operative scientific effort, quickly accomplished. Hundreds of medical scientists in more than a score of universities were employed on numerous investigations under contract with the Office of Scientific Research and Development,

which totaled over $2,400,000. Special equipment, including so-called "altitude chambers" for simulating the effects of high altitudes, human centrifuges for developing great radial accelerations, crash cars for producing high linear accelerations, and devices for inducing motion sickness, were provided for their research. Through a contract between the Office of Scientific Research and Development and the National Academy of Sciences, funds were made available to the Division of Medical Sciences of the National Research Council for bringing into frequent conference groups of scientists working on related problems. Hundreds of detailed reports of the results of research in this field were distributed among the workers, and to the air forces of the Army and Navy and those of our allies. Many of these reports were formal records of work accomplished under government contract with OSRD(CMR) and were accordingly filed through the Records Section of the Committee on Medical Research. Through that section abstracts of pertinent information were given wide circulation in the weekly "Summary of Reports Received by the Committee on Medical Research." Also, because of the close relations between the Committee and the National Research Council, these reports were issued in the Committee on Aviation Medicine series, which numbered more than three hundred separate publications in all fields of aviation medicine and had a circulation of several hundred in this country and abroad.

One of the important reasons for the success of the Office of Scientific Research and Development was the close association of their civilian scientists with the armed forces. In no sector were such intimate contacts more necessary or more effective than among those who were dealing with the human problems of military aviation. Throughout the war, Colonel Lloyd Griffis represented Major General David N. W. Grant, the Air Surgeon of the Army Air Forces, as liaison officer to the Committee on Aviation Medicine. Similarly, Commodore J. C. Adams of the Navy maintained day-by-day contact with the civilian scientists through special liaison officers. The Committee, in turn, kept informed of the needs of the services, not only through these officers but also through the effective contacts of technical aides. Through such strong personal links between the civilian and military organizations, practical problems were quickly analyzed and the results of research in university laboratories were soon translated into new equipment and operational procedures.

This far-reaching undertaking was under the direction of the Committee on Medical Research; the funds for its execution were provided through 69 contracts with 31 universities and hospitals. As has been indicated, the Committee on Medical Research made extensive use of the Committee on Aviation Medicine in the evaluation of proposed projects for research and in the initiation of investigations. This placed increasing responsibilities on the latter committee, so that in the spring of 1942 a series of subcommittees were

constituted to deal with the several major aspects of the field. These activities were acceleration, oxygen and anoxia, visual problems, motion sickness, decompression sickness, and clothing. Although no special subcommittee was created to deal with crash injuries and protection against such injuries, DuBois and Walter F. Miles gave such problems their special attention and sponsored a series of highly successful conferences in that field.

Because of the necessity for frequent operations in Washington, most of these men were from the Eastern States, but much of the aircraft industry and many of the military and naval airfields were located on the Pacific Coast. Accordingly, in order to develop close contact with these establishments and with Western scientists there was created an Advisory Commission to the Committee on Aviation Medicine consisting of eight Pacific Coast scientists. This commission gave invaluable assistance to the Committee on Medical Research in the execution of its program.

By the spring of 1944 the work in this field under the supervision of the Committee on Medical Research had become so extensive that it was deemed desirable to create a Division of Aviation Medicine, with Detlev W. Bronk as Chief, for scientific and administrative supervision of the many contracts.

From the beginning of our activities, American scientists derived much assistance from the Flying Personnel Research Committee of Great Britain and from their Canadian colleagues. Four members of the Committee on Aviation Medicine visited England one or more times during the war, and there was a constant exchange of visits across the Canadian border. By such movements of personnel and the free circulation of reports among these and our other allies, information was quickly disseminated and duplication of effort was avoided.

Detailed accounts of the research in aviation medicine are given in the succeeding chapters. Here it will be sufficient to sketch briefly the scope of that work and to indicate how the many and diverse investigations fitted into a broad undertaking that made possible the human operation of our far-flung aerial campaigns.

One of the most serious obstacles to modern aerial warfare that confronted scientists was a human limitation that appeared early in the history of aviation. In 1862 Glaisher, the English meteorologist, and Coxwell, his balloon engineer, ascended to a reported altitude of 29,000 feet. Glaisher lost consciousness, and both men would have perished had not Coxwell, paralyzed though he was, seized the valve cord in his teeth and released the gas. Man had been freed from his earth-bound existence, only to find that the full utilization of his new machine was restricted by his inability to live at high altitudes.

Bert, the French physiologist, soon discovered that the danger of this new environment was the lack of adequate oxygen. Without a sufficient supply of this gas the nerve cells of the brain cannot carry on their normal activity,

consciousness fails, and death ensues. It was obvious to Bert that fliers who ascend to high altitudes must carry with them a reservoir of oxygen from which they can breathe enough to make up the deficiency in the surrounding air.

These were facts well known to physiologists and to the few aviators who dared flight into the substratosphere, but most of the commercial and military flying in airplanes before the war was at heights where the oxygen in the atmosphere is sufficient to maintain life. Accordingly, even in the late 1930's, we were unprepared for the type of aerial warfare that played so large a role in the defeat of our enemies a few years later.

There were two major aspects of this problem. The first was to devise means for keeping fliers alive and alert at the altitudes to which they were forced by enemy fighters or by anti-aircraft fire. This might be done either by reducing the needs of the body for oxygen or by developing mechanical equipment that would supply the additional oxygen needed. The second difficulty was the subtle prelude to loss of consciousness from oxygen lack. There is an insidious feeling of well-being that quickly lapses into a complete loss of consciousness.

Both the Army and Navy air forces developed extensive programs for instructing flying personnel in the physiological dangers at high altitudes. Many altitude chambers were constructed, and in these the fliers had demonstrated to them the harmful effects of anoxia. Hundreds of aviation physiologists were commissioned for directing these programs and for teaching duties. These activities were military and naval undertakings, but the Committee on Medical Research scientists co-operated in their operation by giving advice and furnishing much-needed information.

During the early years of the war many suggestions were made for altering the normal processes of the body by chemical substances so as to reduce the need for oxygen. The chances of success were not great, but the importance of the problem required the exploration of every possibility. Adrenocortical hormone, ammonium chloride, and methylene blue were among the many substances carefully tested by physiologists. By adding some of these chemical agents to the diet of fliers it was, indeed, possible for them to go three or four thousand feet higher without suffering from oxygen want. But the substances had other less desirable effects, and they were never adequate to take a man to the heights at which many aerial operations were carried on. As is usually the case, it is more feasible to provide by physical means the proper environment for the human body than to alter the course of the normal mechanisms within the body.

Following the former method of attack, Walter M. Boothby and his associates of the Mayo Foundation, shortly before the war, developed a mask that covered the nose and mouth of a flier and thus delivered to him oxygen from a tank carried in the plane. Here was encountered a problem that

frequently arises in technological warfare: the means for providing a reasonable degree of human protection limit the military usefulness of the instruments of combat. To furnish the crew of a large bomber with enough oxygen throughout a long mission requires hundreds of pounds of tanks and accessory equipment. Consequently, for a given design of aircraft the bomb load or the range of operations must be reduced. Accordingly, compromise between tactical requirements, physiological needs, and engineering design became necessary.

The Mayo scientists reduced this difficulty by conserving the oxygen and thus reducing the amount that had to be carried. This was done by placing a rubber bag in the oxygen supply line where it reached the mask. Part of the exhaled air, containing some residual oxygen from the lungs, escaped through a valve; part returned to the bag, where it was mixed with the incoming oxygen from the cylinder and thus conserved the precious supply.

This equipment proved admirable for moderate altitudes. At the higher altitudes to which aviators were ultimately forced by enemy action, there was a danger that in the process of rebreathing the oxygen would be too much diluted and too much expired carbon dioxide would accumulate. Also, the exhaled water vapor froze in the supply lines and valves at high altitudes and blocked the flow of oxygen.

The Navy employed a device designed to conserve oxygen by a similar process of rebreathing, but the exhaled air from the lungs passed through a chemical compound that absorbed carbon dioxide and in doing so produced some oxygen. Here, too, there was danger of freezing in the low temperatures of the higher atmosphere.

The Battle of Britain had given the American air forces a pretty clear idea of the specifications for an aerial war over Germany. High-altitude bombing was one of the essentials, but physiologists and flight surgeons began to wonder whether they were prepared for this with the oxygen equipment that was available.

The obvious need was for a system that would supply just enough oxygen to satisfy the requirements of each flier under any condition of altitude or bodily activity, and do so with certainty. The natural indicators of what that need is at any moment are the nerve cells of the brain that regulate the rate and depth of respiration. A means was already available that controlled the flow of oxygen to the aviator's mask in response to the action of these nerve cells, through the respiratory movements initiated by the cells. This was done by placing in the supply line a regulating valve, which was activated by the suction created by each inspiration.

To conserve the supply of oxygen at low altitudes and to ensure an adequate supply at high altitudes, National Research Council scientists in 1940 designed an oxygen diluter valve controlled by an aneroid. It was thus possible automatically to furnish to the lungs of fliers at any altitude a gaseous

mixture corresponding to sea level or to some moderate, safe level. The feasibility of this device became apparent before it was put into operational use by our air forces, for a similar instrument was found on some captured German planes.

From these beginnings there developed a continuing struggle to improve the oxygen supply systems so that they would, in a more compact and simple form and with less weight, give airmen greater safety in their battles six or seven miles above the earth. To accomplish these ends scores of medical scientists worked in their laboratories or in partially evacuated steel altitude chambers to secure precise physiological data for the designers: the rate of oxygen supply required by the human body, the individual variations in that requirement, the additional oxygen needed at any altitude, the tolerable in-spiratory and expiratory pressures, and the effects of varying degrees of work on the respiratory demands. Much of this important data was finally brought together in a *Handbook of Respiratory Data in Aviation*.

One of the chief requirements for the safe use of oxygen at altitudes above those of 25,000 or 30,000 feet was a suitable means for delivering the gas to the nose and mouth during inspiration. The mask devised by Boothby and his associates was a step in the right direction, but at the higher altitudes there was the threat of freezing and of the inward leakage of oxygen-deficient air. As early as 1940 Dr. Cecil Drinker, of Harvard University, anticipated these dangers and set about devising a suitable mask.

This appeared to be a simple task; actually it was one of the most trouble-some human design problems in aviation. Fliers objected to wearing rubber appendages on their faces. The facial configuration of no two men is the same, and this caused poor fitting and excessive leakage. Freezing of water vapor in the air passages was a persistent problem, the mask and the goggles interfered with each other, and it was difficult to integrate the communica-tions microphone with the mask. Furthermore, how was a man to move about in a bomber when he was connected by a mask and a short rubber tube to a fixed oxygen tank, and how was he to survive during a slow para-chute descent from a great height? To meet these difficulties there were many modifications of the first mask designed by the Harvard group. In their efforts to reduce the dangers from leakage, the Army Air Forces went so far as to employ a group of anthropologists to measure the facial dimensions of more than a thousand cadets. From these measurements five standard types of mask were constructed, and to each airman there was issued one of the appropriate size. Another group, working under the Committee on Medical Research, made a precise mathematical and experimental study of the effects of mask leakage on human performance, under varying conditions of work and altitude. From this came a better basis for safe design and a better definition of the hazards.

Successful as each improvement was in reducing the number of casualties

from oxygen lack, the basic characteristics of the system were unsatisfactory. It was obvious from the start of the war that what was required was a sealed aircraft cabin, in which an adequate supply of oxygen would be maintained by compressing air from the outside with a mechanical compressor. Such a pressurized cabin finally appeared in operational use on the B–29's. Gone at last were the cumbersome oxygen mask, the restriction on free movement, and the bulky clothing for protection against the killing cold of high altitude. The engineers had at last restored to fliers their natural environment while taking them to altitudes unsuitable for life.

But it is only a thin metal shell that separates the friendly atmosphere within the cabin from the old dangers that lurk outside, and it is a wall readily pierced by enemy missiles. Suddenly the precious oxygen may be lost; in a brief second the crew may be exposed to a greatly reduced pressure. Here, then, were possible hazards to the fliers.

Physiologists readily determined the length of time men could survive the loss of oxygen, and emergency masks and supply systems were provided. But there was little knowledge of the effects of quickly lowering the pressure on the human body. Normally there is an equalization of pressure inside and outside the body; the amount of gas in solution in the body fluids depends on the external pressure. How a violent change of pressure from that of sea level, maintained within the cabin, to that of 40,000 feet, outside the cabin, would affect men was a problem that the Committee on Medical Research requested physiologists to investigate.

To do this, the investigators evacuated one section of a steel altitude chamber with a suction pump to a pressure corresponding to a possible flying altitude. Another smaller section, containing the subjects of the experiment, was kept at sea-level pressure. Between the two was a thin wall. When this wall was suddenly ruptured, there was in the second section of the chamber a precipitate fall of pressure. Thus it was found that objects in the path of the air flow to the hole were violently disturbed, and subsequently there has been at least one instance of a flier's being blown out of a ·ruptured port in a B–29. But the experiments showed that there are no harmful effects within the body resulting from the sudden change of pressure. Later, Army Air Forces physiologists safely decompressed themselves at a rate of 8000 to 30,000 feet in one hundredth of a second. From such evidence came the assurance that the physiological advantages of pressurized cabins are not offset by physiological dangers.

The height to which men can go in these cabins is limited only by the skill of the aircraft designer. This is not so for men flying with the aid of an oxygen supply system. Above 38,000 feet the barometric pressure is so low that insufficient oxygen goes into the blood passing through the lungs, even though pure oxygen be delivered to the mask. To satisfy the human requirements at these great heights, oxygen must be delivered under a

pressure sufficiently high to load the blood adequately. Before the advent of pressurized cabins, there was an uncomfortable possibility that this physiological limitation might determine the outcome of the three-cornered race for altitude between bombers, fighter-interceptors, and anti-aircraft fire.

The Germans sought to meet the difficulty by getting their interceptors up to 40,000 or 45,000 feet with jet propulsion and down again so quickly that the pilot would survive the oxygen lack. As a temporary expedient, physiologists of the Allied air forces modified the mask and regulating valves of the usual oxygen supply systems so that the pressure within the mask was greater than that of the ambient air. Thus more oxygen entered the blood, and the top level of operations was increased by some thousands of feet. The achievement of this result posed a long series of physiological questions, such as the effects of the increased pressure on blood flow through the lungs and the relative advantages of a constant high pressure or an intermittent pressure that varied with the respiratory cycle.

Few fliers have experienced the explosive decompression of a pressurized cabin, but it is not unusual in modern fighter aircraft to climb at a rate of 80 feet a second and to reach within six minutes an altitude of 6 miles, where the barometric pressure is but one-third that at sea level. Even this change in the pressure acting on the body unbalances the equilibrium of gas pressures within its cavities and tissues. Indeed, the physiological consequences can be more harmful than those resulting from the very rapid pressure changes of shorter duration already mentioned. The painful inward pressure on the eardrum when the eustachian tube cannot be opened is a familiar experience of all who have flown, but only the military aviator who goes quickly to 30,000 or 40,000 feet knows the excruciating pain caused by the sudden liberation of gases from solution in the blood or other body fluids.

This was not an unknown problem before the days of fast fighter craft. Divers had experienced the painful consequences of coming out of the high pressure in a diving bell into the lower pressure of atmospheric air. To those symptoms physicians had given the names caisson disease or bends or decompression sickness, and physicians concerned with diving operations had studied their cause. There was little question that the pain was in some way the result of gases coming out of solution in the fluids of the body. Beyond that our knowledge was scanty.

The prospect of pilots and air crews writhing with the pain of bends at 35,000 feet did not augur well for the success of aerial warfare, and yet there was the possibility that we should have to fight at those altitudes. Accordingly, the Committee on Medical Research supported many investigations under the supervision of the Subcommittee on Decompression Sickness. Because of their research we know a good deal about the causes of bends in aviators, the frequency with which bends occur, and the means for preventing them.

Several years were spent in uncovering the sequence of events that takes place. It was found that during the sudden changes of pressure minute gas nuclei on the surface of cells or on the inner walls of blood vessels rapidly expand in size, growing with the nitrogen, carbon dioxide, and oxygen that are liberated from the surrounding fluid as it is decompressed. As bubbles of gas are thus formed and grow, some lodge in small terminal vessels, where they obstruct the flow of blood. Nerve endings may thus be deprived of oxygen, and pain results. Or regions of the brain are likewise put out of action, with widespread and serious consequences.

These observations and the resulting theory of decompression sickness led to a practical and fairly reliable method of prevention. Assuming that the bubbles are largely composed of nitrogen — this being the most plentiful source of gas within the body fluids — it was suggested that the supply of nitrogen for bubble formation could be reduced by breathing pure oxygen for some time before a flight. Nitrogen in the tissues is thus replaced by oxygen. Because the oxygen is consumed by cellular metabolism, and because its tension in the blood falls rapidly when a little is removed, it is less potent as a source of bubbles. This was proved experimentally, and the practice of breathing oxygen before a flight or on the climb to altitude is now a proved but not infallible means for preventing decompression pains.

Actually, the incidence of decompression sickness among fliers was never very great. In large measure it was due to the fact that men were rarely at a "bends altitude" of 35,000 feet or more for long periods of time. But if operating conditions had been less favorable in this respect, we were prepared to meet the problem.

Radar- and radio-controlled projectiles could keep man out of the air in a future war. But World War II has been fought by men with the aid of their senses; indeed, the instruments that have supplemented the senses have at the same time made greater demands on them. Take the matter of night flying, for instance, which has been made possible by instrumental devices. The strategy and tactics of aerial war have thus been greatly extended, for the airman can now utilize Nature's most effective form of camouflage. Unfortunately, this advantage is also available to the enemy, so that there is required a keen ability to see through darkness the dim form of an enemy aircraft or the contours of an unlit airfield.

To watch a night mission return to blacked-out Britain was to appreciate how unnatural such duties were, and how difficult, for the young airman who had grown up with a light switch at his fingertip and a flashlight in his pocket. Nor were there many physicians who knew enough about night vision to be helpful with advice on seeing at night. Few remembered the elementary facts that the cone cells of the retina, which are used in day vision, do not respond to dim lights; that the rod cells, which are used in night vision, are located in the peripheral regions of the retina, so that one

sees a dim object best by looking a little away from it; and that the sensitivity of the rods is destroyed for some time by a bright light, and is least affected by red of all the colors. And yet the translation of those principles into tactical practice was an important element in the success of night operations.

Because of the great military advantage of keen night vision, there were many suggestions for improving the ability to see at night by the use of chemicals and drugs. The most promising of these agents was vitamin A. The basis for this suggestion was the discovery by physiologists that a deficiency of this vitamin in the diet causes defective night vision. During the years before the war Hecht and Wald had thoroughly investigated its role in the visual process. As the pattern of our allies' war unfolded and revealed the importance of night combat, scientists carefully tested the night vision of thousands of young airmen and sought to improve it by the administration of large quantities of vitamin A. As had been learned before, they found that inadequate diets caused partial night blindness, and this was an important fact for the flight surgeons who cared for the health and efficiency of our fliers. But the administration of extra quantities of the vitamin was without benefit; nor was any other substance found that would improve night vision.

Although no means were found for improving the natural physiological endowment of airmen for seeing at night, it soon became apparent that much could be done to protect vision and to use natural abilities more effectively. One of the first steps toward this end was a revision of lighting practice on airfields and in aircraft. Following prolonged exposure to bright light, the eyes require about half an hour in which to become dark-adapted; until then night vision is below normal. Accordingly, a pilot who turns his gaze from a lighted instrument panel out into the darkness, where enemy planes are hidden, can see but poorly for some time. To minimize this disadvantage the Committee on Medical Research sponsored the design of a physiologically acceptable lighting system for aircraft enclosures, which has as its principal feature the use of an appropriate wave length of red light. This practice, which was adopted by the Navy, makes possible cone vision for precise observations within the aircraft, without affecting appreciably the subsequent sensitivity of rod night vision for distant, dimly illuminated objects.

Reasoning along these same lines, red goggles were designed that could be worn by airmen prior to night flight. With this physiological aid they were able to go quickly into combat without the handicap of meeting, relatively blind, a dark-adapted foe.

Typical of the new problems that were created by the unfamiliar environments of world-wide war was the prolonged impairment of night vision discovered by a visual expert among personnel on the glaring beaches of the tropics. There the visual purple of the retina was so much bleached by the sunlight that the ability to see in dim illumination was depressed throughout

the night. To avoid this previously unrecognized handicap, the use of appropriate sun glasses was recommended to our fliers.

The oxygen-deficient atmospheres of high altitudes were also environmental causes of poor night vision. Careful measurements showed that night fighters at 5000-feet altitude could see less well than at ground level, and that their vision deteriorated steadily as they went higher. After laboratory tests revealed the seriousness of this handicap, military regulations were established that required all airmen to use at night an auxiliary supply of oxygen, even at moderate altitudes. Only thus was it possible for them to take full advantage of their normal visual powers.

A second important activity was the formulation of a campaign to teach airmen how to see at night. Fortunately there were both precedent and personnel for this undertaking. Both the Army and Navy Air Forces, from the beginning of the war, had found it necessary to teach their student fliers a good deal of physiology, so that they would understand the effects of high altitude and little oxygen on their bodies. Hundreds of physiologists were commissioned as instructors, and these young scientists taught the future fliers the rudiments of human biology, revealed to them the symptoms of oxygen want in partially evacuated altitude chambers, and instructed them in the use of protective oxygen equipment. After convincing the military authorities that the ability to see at night could be improved by knowledge and training, our visual experts turned to these aviation physiologists for assistance in disseminating the information about night vision that had been learned through years of research.

Military aircraft are designed to move men rapidly, but some of the movements men undergo in aircraft are not desired, and some are harmful to the human body. Among these are the motions of a plane caused by air currents or rough air. They are relatively harmless, but anyone who has experienced the nauseating symptoms of motion sickness will agree that they could seriously reduce the fighting efficiency of an airman. When large numbers of unseasoned fliers were recruited this condition became a threat to the effectiveness of aerial operations. Accordingly research was directed to the discovery of the conditions most likely to cause motion sickness or drugs that would prevent it.

One group of investigators tried to determine the type of motion that is most objectionable. A "nauseator," not unlike an elevator, was constructed, and in this device men were moved through space — up and down at varying speeds, through various distances, and with different accelerations. Ultimately the results of such tests should provide information that will enable aircraft designers to construct planes that are freer of the movements responsible for motion sickness. But this is a long-range undertaking. Begun under the emphasis of a war-accented need, the work can best be carried forward in the more deliberate spirit of peacetime research.

In the course of such tests, information was also gathered concerning the type of person most likely to experience motion sickness. Certain mental characteristics were found to be predisposing factors. Also, habituation to the characteristic motion of an airplane was an excellent preventive of nausea. In accordance with these observations, the air forces found a remarkably low incidence of motion sickness among their flying personnel, presumably due in part to the type of men selected for flying duties and in part to the fact that they were thoroughly accustomed to the movements of aircraft.

The situation was not so favorable among ground troops, who were only occasionally carried by glider or aerial transport. There was a similar contrast between seasoned sailors and soldiers transported by landing barges or other vessels used in amphibian operations; in both cases the foot soldier was much more disposed to motion sickness. To protect these men, vigorous efforts were made to find drugs that would prevent the nausea. Many searching tests were made of the effectiveness of various drugs and of the efficacy of different bodily postures, especially in the course of amphibious training operations. No new drugs of value were discovered in the haste of the war effort, but improved combinations of previously known drugs were found to be effective in about 70 per cent of persons tested who would otherwise have suffered from motion sickness.

There is another type of harmful movement in military aircraft that is essential for good fighter planes. Their high speed and great maneuverability enable them to excel in plane-to-plane combat, to evade the heavier fire power of larger craft, and to give effective protection to bomber missions. Engineers and metallurgists worked for years to develop planes that would withstand the centrifugal forces of high-speed turns and "pull-outs," but during that time there were no corresponding improvements of the physiological characteristics of the men who were to utilize the new machines during such maneuvers.

A normal heart and circulation will deliver enough blood to the brain when the body is erect or recumbent, and will meet the needs during sudden changes in posture. Messages from pressure-sensitive nerve endings in the walls of certain blood vessels promptly report to the nerve centers regulating the heart and blood vessels a drop in blood pressure within the vessels supplying the brain. The effect of this is an accelerated heart rate and a constriction of peripheral vessels. Thus the circulation of the brain is again increased. But the cardiovascular system and this reflex control were not evolved for pumping blood made five to ten times heavier by a suddenly applied centrifugal force. Yet such forces do act on a fighter pilot as he makes a steeply banked turn at high speed or pulls out of a power dive.

The most valuable instrument for this research was the human centrifuge. A long horizontal arm, rotating about a vertical axis, carried at the outer end a seat for the subject. A man sitting in this could be subjected to centrifugal

forces of a desired magnitude while various physiological reactions were measured. Following the example of the Royal Canadian Air Force, which had done valuable pioneer work in this field, two such devices were built by the Committee on Medical Research in civilian laboratories within the United States. The first was at the Mayo Foundation, the second at the University of Southern California.

Under these controlled conditions, men were rotated so that they were under the influence of a centrifugal force five to ten times as great as the force of gravity. When this force was in the direction of the subject's feet, as is the case in most high-speed aerial maneuvers, "gray-out," then blackout, of vision were the first effects. If the centrifugal force was sufficiently great and was prolonged for some seconds, loss of consciousness followed. Measurements of heart action and of blood pressure in various parts of the body during rotation showed that the heart was unable to pump enough blood to the brain, which was thus deprived of the oxygen needed for carrying on its normal activity. With smaller centrifugal forces there were no obvious symptoms, but often-repeated reductions of the cerebral blood flow ultimately caused fatigue, irritability, and inefficiency.

Three methods were devised for aiding the heart to overcome this handicap imposed by swift combat planes. One was to place the pilot in such a position that the centrifugal force acted at right angles to the axis of his body. Under these circumstances the heart is not required to pump the blood against the centrifugal force. In the centrifuge this was readily accomplished by holding the subject's body perpendicular to the rotating arm. In a plane it would be necessary for the pilot to assume a prone position. This would have required a radical redesign of the aircraft and its controls, as well as the technics of operation. Accordingly, this procedure was not employed. Some steps were taken in this direction, however, by providing elevated foot rests and provisions for crouching so that as much of the pilot's body as possible was perpendicular to the path of the plane during the turn or pull-out. Greater accelerations were thus tolerated.

A second procedure derived from these physiological experiments was that of tensing the muscles of the abdominal wall and of the lower extremities with a closed glottis so as to aid the return of blood to the heart and thus circulate more blood to the brain.

The physiological basis for the effectiveness of this procedure was also the basis for the "anti-G suit." This was the most useful aid to fighter pilots in overcoming blackout. At least three civilian groups working in collaboration with service laboratories and those of our allies made important developments along these lines. In each case the basic concept was the same. By a constant force applied to the surfaces of the arms and legs and abdomen, or by pressures that increased as the centrifugal force increased, excess blood was prevented from accumulating in those regions. Thus the normal flow of

blood to the brain was better maintained. With the aid of these suits, pilots retained their normal faculties under the action of centrifugal forces several times greater than a force that would otherwise have produced a temporary loss of consciousness. The safety and efficiency of pilots in aerial combat was thus increased.

The severest forces of acceleration or deceleration to which an airman is subjected are those that occur during aircraft accidents. A plane that crashes into the earth or another object may decelerate in a few seconds from a speed of hundreds of miles an hour to rest and thus exert tremendous forces on objects free to move within it. Thus the bodies of the crew continue to move forward until they strike a fixed portion of the plane or the earth.

The avoidance of the human consequences of such forces has been the concern of engineers, training officers, and flight surgeons, but there had been little systematic and co-ordinated effort by biologists and engineers to deal with the basic aspects of this problem. To meet this challenge, a comprehensive program of research was framed under the direction of DuBois. It had three objectives: determination of the causes of aircraft accidents and the nature of the resulting injuries; the analysis of the structural characteristics of the plane responsible for the injuries; and the redesign of seats, safety harness, control panels, escape hatches, and other parts of the plane so as to reduce the likelihood of accidents and minimize the injuries resulting from unavoidable accidents.

Through collaboration with the Office of the Air Surgeon of the Army Air Forces a detailed survey was made of the frequency of various injuries and their relation to the type of plane, the structural portion of the plane causing the injury, the training experience of the pilot, and the operating conditions at the time of the accident. This information suggested modifications in training practice and in operational procedures that increased the safety of flight.

Of more far-reaching significance was the revelation of unnecessary structural characteristics that were frequently responsible for injury. Safety harness was improved, weak seat supports were strengthened, and sharp projections were removed from in front of the pilot's head. To test the potential danger of these causes under controlled conditions and to assess the value of improvements there was constructed at Cornell a crash car that could hurtle a dummy pilot and its protective equipment swiftly into an obstacle. From these and similar studies there began a movement for greater safety in aircraft, which is continuing into the peacetime aerial age.

THE STUDY OF CRASH INJURIES AND PRE-VENTION OF AIRCRAFT ACCIDENTS

EUGENE F. DU BOIS

IT IS the purpose of this chapter to outline the methods by which a civilian group acting in close co-operation with the armed services helped to reduce the number of crash injuries. In the course of this study much information was obtained concerning the defects in aircraft equipment and design responsible for these injuries. It soon became apparent that it was necessary to concentrate on the causes of accidents and to determine scientifically the factors included in the loose term "pilot error."

The Committee on Aviation Medicine of the National Research Council first undertook the study of flying accidents in May 1941, and developed the work more intensively as it later came under the guidance of the Committee on Medical Research. In 1941 the number of crashes and injuries was appalling, and indeed throughout the war the fatalities in training exceeded those in combat. The military authorities were fully aware of the importance of the subject, but there was little basic information available.

Our own stimulus came directly from Hugh De Haven, who had been trying for many years to induce various other organizations to initiate a study of crash injuries. De Haven while in training as a pilot in World War I had survived a midair collision in spite of multiple serious injuries. In March 1941, he had published an article entitled "Miraculous Safety" describing a series of survivals in persons falling or jumping from great heights. It was evident from his analyses that survivals were due to the absorption of deceleration by yielding structures, such as six inches of soft earth or even the top of an automobile. It was also evident that the human body could withstand surprisingly high forces. De Haven reasoned that many lives could be saved in crashes where the forces encountered were understood and the pilots were given proper protection.

The Committee on Medical Research, acting on recommendations from the Committee on Aviation Medicine, furnished prompt support and started a research project under De Haven in Cornell Medical College in New York City. The Civil Aeronautics Board at once became interested and began to send in the reports of crashes in civilian light training planes. Its engineers noted the cause of each accident and the landing conditions and described or

photographed the wreckage, and the local medical representative gave an account of the injuries. At first the reports were incomplete, but when the doctors found that a medical college was making a scientific study and that it was not mere government routine, they began to co-operate with considerable enthusiasm. Attention was concentrated on crashes in which there was serious or moderate injury and a chance of survival, since there was no point in analyzing trivial accidents or crashes of such severity that the plane was completely disintegrated.

It was apparent that the vast majority of the serious injuries sustained involved the head and face. Many of these were due to breakage of the seat or seat belt or its attachments. Even if these held, the pilot or passenger would catapult forward and his head would strike an instrument panel close to the seat, rigidly constructed and bristling with projections. Some injuries were caused by struts, others by poorly designed control wheels. Legs were fractured by rudder pedals that resembled ankle traps. Each type of plane had its own pattern of injury. There was practically no evidence of abdominal damage from belts, and there were surprisingly few serious fractures of the spine.

As evidence of this nature began to accumulate, the Committee on Aviation Medicine was able to plan its campaign in co-operation with the Bureau of Medicine and Surgery of the Navy and the Air Surgeon of the Army Air Forces. It employed Dr. B. Audrey Schneidst, a statistician, to work on a system of crash reports in the Office of the Air Surgeon, and the Navy instituted a system of records of crashes similar to the one devised by De Haven. Boards containing flight surgeons were organized at Army and Navy training stations. The Office of Flying Safety of the Army Air Forces at Winston-Salem, North Carolina, and the corresponding division of the Navy took a part in the study. Experimental studies were developed at Wright Field, Randolph Field, the Navy Medical Research Institute, and the Aviation Section of the Research Division of the Bureau of Medicine and Surgery.

Meanwhile it was necessary to secure the co-operation of the aviation industry. It would have been possible to conduct a publicity campaign in the press with articles similar to "And Sudden Death," which created a stir among automobile manufacturers. This type of action, however, would have caused apprehension among the friends and relatives of fliers and would have embarrassed the manufacturers, who could not have rebuilt their planes on short notice.

During 1942 there were relatively few civilians or officers working on crash safety, but by the spring of 1943 several groups had been organized in different parts of the country. The Committee on Aviation Medicine, in co-operation with the Navy and the Air Surgeon's Office, arranged an informal meeting at the National Research Council Building on May 27, 1943. This was the first of five so-called Crash Injury Conferences, which grew

in size and importance. Inasmuch as they were informal and without direct authority, they served as a forum where junior officers could express their views freely. Since they were attended by many representatives of each group working on the subject, they made it possible to co-ordinate the efforts of all the investigators. Liaison officers from the air forces of Great Britain, Canada, and Australia kept us in touch with the Allies. There was a common enthusiasm and a common impatience over delays. All new ideas and devices were reported promptly, and no one cared about individual credit.

At the time of this country's entry into World War II, there was a curious reluctance on the part of our fliers to use shoulder harness. The British fliers fully appreciated its value and consistently wore the Sutton harness (which incidentally had been developed in California). The lap belt is not nearly so efficacious in case of crash, because it allows the pilot to jackknife forward and strike his head on the instrument panel. On the other hand, it does not cause the constraint imposed by shoulder harness. There were hundreds of unnecessary injuries before shoulder harness became mandatory in military planes, and there are still hundreds among civilian fliers, who seem to disregard shoulder harness entirely.

An ingenious mechanism intended to snap a protective device into position in case of crash was invented by De Haven. Inside a 6-foot tube of diagonally woven nylon he placed a second rubber tube containing a train of special powder. When this was ignited by a fast squib, the tubes would expand in a few thousandths of a second and in their expansion would contract 18 inches. Such a tube, weighing a few ounces, could jerk a weight of 150 pounds 10 feet in the air. This device was never used, because Dr. William A. Geohegan of Cornell Medical College, working on the same project, made a striking improvement in the mechanism of the shoulder harness. He devised an inertia lock that would on any impact greater than $2\frac{1}{4}$ g [1] lock the wire cable that restrained the shoulder harness yet release it the instant the force terminated. Repeated tests on dummies showed that it gripped the cable before it had traveled more than $\frac{1}{2}$ inch, but there was a stretch of a few more inches in the harness. The previous mechanism had been controlled by an awkward hand lever, which was often forgotten by the pilot in case of impending crash.

Over a hundred of these locks were made in the Cornell apparatus shop and sent to the Army, Navy, and Royal Air Force. After a long delay they were adopted by our armed services in a slightly modified form. They are now standard equipment; they are comfortable and automatic and are saving lives.

In the meantime, the study of crash injuries in light training planes had developed certain facts that were found to apply to small and medium-sized military aircraft. In his first report, made in November 1943, he made

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[1] This abbreviation represents the force of gravity multiplied by the figure given.

an analysis of thirty survivable accidents and drew a diagram (Fig. 25) showing that injuries to the head were dominant, followed by injuries to other peripheral parts, with relatively infrequent damage to the lower half
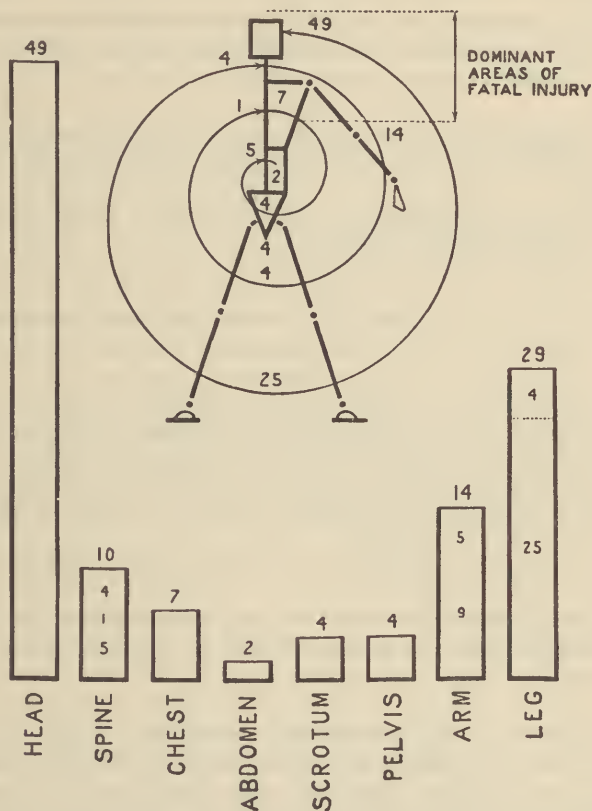
FIGURE 25. *Diagram showing the peripheral trend in frequence of principal injuries caused by airplane crashes.*

of the torso. The same trend has been noted in other analyses. This first report and the second, which was made in July 1945, are interesting as showing a scientific method of approach to a new problem.

The older methods of reporting injuries in three or four categories proved unsatisfactory and a ten-degree scale estimate of degrees of injury was devised. This made it possible to compare the force of the crash and damage to the plane with the extent of injury to the occupants, and it became apparent that certain planes or positions in certain planes had unusually high

scores for damage. For example, in many accidents one of the two occupants would be severely injured while the other suffered but little. Every accident that was properly reported added to the store of knowledge and taught a lesson in the prevention of injuries.

De Haven's second report was based on an analysis of 110 accidents in fore-and-aft seating and 75 with the side-by-side seating arrangement. We cannot do better than give his conclusions:

(1) In accidents where cabin structure is distorted but remains substantially intact, the majority of serious and fatal injuries are caused by dangerous cabin installations.

(2) Crash force — sufficient to cause partial collapse of present cabin structure — often is survived without serious injury.

(3) The head is the first and often the only vital part of the body exposed to injury.

(4) Fundamental causes of head injury are set up by heavy instruments, "solid" instrument panels, seat backs, and unsafe design and arrangement of control wheels.

(5) The probability of severe injuries of the head, extremities, and chest is increased by failure of safety belt assemblies or anchorages. In one type of aircraft studies, safety belt failure occurred among 70 per cent of the survivors.

(6) Failure of the 1000-pound safety belt occurred in 94 cases among 260 survivors. Only 7 survivors showed evidence of injury to abdominal viscera: 2 of the injuries were classed as serious.

(7) The tolerance of crash force by the human body has been grossly underestimated.

(8) If spin-stall dangers are lessened and safer cabin installations are used, fatal or serious injuries should be rare in the types of aircraft studied — except in extreme accidents.

The thirteen recommendations made in this report included strengthening of cockpit structure, of seats, and of belts. De Haven advised redesign of the instrument panel so that it would not fracture skulls, and of control wheels so that they would cushion impact instead of crushing the chest. In conclusion he cited the experience of stunt pilots who made deliberate crashes for the moving-picture industry and county fairs. One had made ten intentional and fifteen unintentional crashes without serious injury.

The development of the campaign for air safety is best shown by an account of the five Crash Injury Conferences mentioned above. The first was attended by twenty-eight civilians and officers, the fifth by over one hundred and fifty. It is interesting to note how this movement, which started with an effort to reduce the severity of injuries in crashes, expanded into the whole field of the causes of crashes, their prevention, and the larger aspects of flight safety. It is significant that although the meetings were entirely informal, the influence of the group was so strong that most of the recommendations of

the third conference were adopted by the military services as though they were official.

At the first Crash Injury Conference, held in May 1943, reports were made of the pioneer work of Lieutenants Dietz and Dobbs of the Naval Air Station, Pensacola. Other speakers discussed the activities of the Flight Control Command at Winston-Salem and the difficulty in getting the training command to use shoulder harness. The meeting ended with a report from De Haven on seventy-three survivable crashes.

The second conference, held on January 19, 1944, was devoted to a series of papers on statistical studies, accidents in the Army and Navy, and methods of protection. Captain George W. Hass of Randolph Field gave a comprehensive analysis of autopsy findings in aviators killed in crashes. He emphasized the difficulty of bailing out of a spinning plane and suggested the desirability of some mechanism for ejecting the pilot. Dr. Geohegan and Dr. Hinsey described the new automatic lock.

The third conference, held on May 15 and 16, 1944, was devoted to the subject of the safer cockpit. This meeting was by all odds the most important of the whole campaign. The recommendations were so clearly expressed that they guided the program for the next two years.

This conference was divided into several small groups, which prepared reports with recommendations. Each approached the subject of the safer cockpit from a different point of view, but there was general agreement regarding the main objectives and manner of attack.

The group on seats and harness recommended that much more attention be given to comfort and that the entire cockpit, including the seat, be co-ordinated with the desired optimal position of the pilot. Inasmuch as accidents due to too strong a seat were rare and accidents due to weak harness and seats were common, it was considered advisable to strengthen both to withstand impacts of 40 g. It was also recommended that the Geohegan type of inertia lock for shoulder harness be adopted.

Other groups covered aircraft armor in relation to cockpit structure and fire hazards and escape hatches, matters of extreme importance in crashing and ditching.

The group on injury hazards summarized the large amount of accumulated knowledge and made eighteen recommendations for changes in structure and equipment. It reported that "pilot error" was said to be responsible for 76 per cent of the fatal naval aircraft accidents in training and for 58 per cent of those in operational flying.

The group specifically discussing pilot error recognized the many factors included in this loose term and concentrated on those that could be reduced or eliminated by changes in the cockpit. It was recognized that many accidents were due to the pilot's confusion of controls that were badly located. For example, in some planes the handles for landing gear and flaps were

side by side and identical in contour. There was no good reason why they should not be spaced far apart and given characteristic shapes of handles that would indicate their nature, even to the gloved hand. Except for the most important dials and controls, there was little or no standardization in the different aircraft. The group recommended a careful study of actual sequences of movement and the optimal design of instruments, dials, and control mechanism. They insisted that the work-place layout must provide for ideal motion sequence of eyes, hands, and feet, plus the consideration of perceptual processes. On the basis of these principles it was recommended that all flight-control instruments be given standard geometrical locations.

Instruments and controls were considered in detail by a group led by Lieutenant Commander Norval R. Richardson, who had long been working on the subject of standardization in Army and Navy planes. There was still a difference of opinion on the arrangement of the six basic flight instruments on the panel. It was agreed that the most vital factor in instrument arrangement was standardization, which was considered more important than arrangement itself. The group recommended that instruments and controls be arranged primarily for the convenience of the pilot and only secondarily for ease in maintenance and manufacture. In general it was agreed that it was highly desirable for controls to travel in the same direction as the equipment they operated. Finally, a careful study was suggested of existing mock-ups of cockpits, especially the new types already started at the Special Devices Division of the Bureau of Aeronautics, along the principles advocated at this meeting.

The group on aircraft design, manufacture, and maintenance came to conclusions similar to those of the other groups. It stressed the desirability of developing a geometric area that in the event of a crash without belt or seat failure would be free of obstructions that a pilot's body might strike. It suggested the adoption of the console type of switch mounting, which could be folded out of the way when not in use. It was agreed that instrument standardization should be carried much further and that all controls should be functional in their motions. Study of the work already done on standardization and functionalization of controls and instruments was recommended.

At the final meeting of this conference, the reports of all the groups were discussed. Plans were made for the dissemination of information and further study, particularly in the psychology of the pilot in using his instruments and controls. It was hoped that more mock-ups of ideal cockpits would be built and exposed to criticism.

It must not be supposed that a large conference could be so well co-ordinated without a great deal of preliminary planning. As a matter of fact, a start had been made on the so-called "ideal," or standard or functional, cockpit at the Special Devices Division of the Navy. The story of this development is not without interest in view of its great expansion. It is an

example of what can be accomplished by the co-operation of medical and line officers.

There was always a tendency on the part of line officers to consider that the duties of medical officers were limited to health and sanitation. During World War II, however, it became evident that medical men could help in removing some of the physiological limitations that interfered seriously with operation, particularly in flying. By the end of 1943 Captain J. C. Adams, in charge of the Aviation Section of the Bureau of Medicine and Surgery, had established the best type of co-operation with the key Military Requirements Division of the Bureau of Aeronautics. Officers assigned to this liaison proved in a tactful manner that experienced medical officers could be of great practical service in the matter of equipment for the pilot. Being in close touch with the work of the Committee on Aviation Medicine, Captain Adams and the assigned officers helped to develop in the Military Requirements Division a plan for the construction of an "ideal cockpit" and arranged to have the work done in the Special Devices Division.

The Special Devices Division was accustomed to devising new apparatus and was expert in making mock-ups of cockpits. A wooden cockpit was soon ready with its comfortable seating, simplified design, and functional arrangement of controls. This mock-up was exposed to large numbers of pilots and engineers and was modified in a hundred details before the Military Requirements Staff recommended its adoption for fighter planes. As the work expanded it became one of the major efforts of the Special Devices Division.

By the time of the fourth Crash Injury Conference, held on February 21 and 22, 1945, great advances had been made, and at this meeting there were available several mock-ups of the Navy "functional cockpit" and one made by the Chance Vought Aircraft Division of the United Aircraft Corporation. This conference was attended by several representatives of manufacturers. Substantial progress was reported. It was stated that cockpit standardization had been placed on a working basis by means of various committees, and extended to British aircraft as well as that of the Army and Navy. An Army-Navy civil committee had been established to standardize cockpit controls for military aircraft to be used in civilian aviation.

The importance of comfort in seats was becoming more and more apparent, since the extended ranges of aircraft were such that the endurance of the pilot was the limiting factor. The desirability of strengthening seats and harness to withstand 40 g was reaffirmed, although it was conceded that this figure was only an estimate, lacking an experimental basis. The conference discussed the question of making important tests by deliberately crashing worn-out aircraft from catapults or by other means.

In the meantime, the crash injury project at Cornell Medical College had opened a small research station in Washington. Its work was chiefly directed toward future developments in jet and other high-speed planes, with a con-

sideration of new instruments and safety devices and the psychology of pilots in relation to cockpits. By this time both branches of the armed forces had developed well-equipped research stations in the field of flying safety. The Army Air Forces was active in psychological investigations, and the Navy was undertaking research in motion-time studies. Somewhat later the Cornell project was broadened to include a study of helicopters under a test pilot and an industrial designer.

The fifth and final conference, held on October 30 and 31, 1945, had so broadened in scope that the name was changed to Flight Safety Conference. There was described a study of impact forces on human beings and the new type of harness that would permit the body to stand terrific impacts. Another group devoted its sessions entirely to helicopters. A careful analysis was given the many defects of the present helicopter cockpits, and the possibilities of helicopters and the desiderata were explained. Possible changes were outlined and sketches of modified cockpits and improved control wheels were shown. This meeting was attended by representatives of manufacturers of rotating-wing craft, who took an active part in the discussions.

The second meeting of this conference was devoted to the many new problems presented by jet planes, such as vision, cockpit temperature, the gravity forces in turns, and the effect of the pilot's position. Work with the centrifuge at the Mayo Foundation had shown that a pilot exposed to $2\frac{1}{2}$ to 3 g can hardly move, much less bail out. Great emphasis was placed on the need for being able in case of accident to eject the pilot with his seat a sufficient distance to clear the tail of the jet plane.

All projects were terminated on December 31, 1945, but means were found by the National Research Council to continue De Haven's crash injury studies in the Department of Physiology of Cornell Medical College.

During the whole period of the war the project was in close touch with the British and Canadian air forces and later with the Australian also. Their liaison officers added much to all the meetings. The Royal Air Forces made magnificent contributions in the field of air and sea rescue, and many of the reports of their flight surgeons on head and spinal injuries will remain classics. Professor Bartlett, a psychologist from Cambridge, England, and his associate Craik were pioneers in their study of instruments and controls. Craik's death in a motor accident robbed us of one of the most brilliant young investigators.

The Royal Air Forces Medical Laboratory at Farnborough devoted a large portion of its efficient work to air safety. It made careful analyses of forces and injuries. One of its staff devised a "cell" or hammock that could be used in case of impending accident to distribute impact loads safely on the body. In various modifications this principle should be of great service.

There is no question regarding the improvements of air safety and the saving of many lives. At the present writing (July 1946) the movement is

going ahead rapidly, particularly in civilian aircraft. More and more of the new planes have cockpits incorporating the recommendations of the crash injury project. The project itself is now being continued under the National Research Council, with financial support promised by the Army and Navy, the Civil Aeronautics Authority, the Aircraft Industries Association of America, and the Aircraft Owners and Pilots Association. The Army and Navy are actively engaged in research, and it is believed that they will soon be able to study crashes of old airplanes made by means of catapults or fast-moving platforms. There are still many problems to be solved.

Unfortunately it has not been possible to give credit herein to several hundred officers and civilians who made important contributions to the study of crash injuries and air safety. Most of their names have appeared in the reports of the Crash Injury Conferences. Two brilliant and devoted pioneers in this field, Commander Eric Liljencrantz (M.C.) USNR and Lieutenant Colonel M. W. Boynton (M.C.) Army Flight Control Command, lost their lives while making hazardous experiments in their endeavors to promote the safety of others.

CHAPTER XXI

THE EFFECTS OF ACCELERATION AND THEIR AMELIORATION

EUGENE M. LANDIS

I N THE struggle to obtain tactical advantage in aerial combat, engineers have been able to supply modern armies with planes that can reach peak speeds of almost 600 miles per hour, or ten miles a minute. Even as this chapter is being written, new tests with jet-propelled planes may well attain even higher speeds. Sustained velocities of this grade have so far not produced any symptoms in pilots protected by a closed cockpit so long as the path of flight is straight, but as soon as the direction of flight is altered both the plane and its occupants are subjected to stresses of high order.

The importance of these stresses first became evident as pilots trained for the Schneider Trophy races of 1929. At this time pilots described dimming or complete loss of vision when making sharp turns at high speeds. The experiences of Navy pilots during training led to the pioneer studies in this new field of applied physiology by Captain John R. Poppen, beginning in 1932. With the advice of Professor Cecil K. Drinker of Harvard University, Captain Poppen measured, by a special manometer, the arterial blood pressure of dogs exposed to the stresses of dive-bombing maneuvers in planes. His report in 1935 not only demonstrated the fundamental nature of the circulatory effects of radial acceleration but also suggested the use of a pneumatic belt for protection. This study was followed in 1937 by a report from Wright Field describing tests in which animals and men were exposed to radial acceleration in planes and also in a centrifuge having a radius of 10 feet. Since 1933 numerous papers on acceleration have appeared in European scientific publications relating to aviation medicine.

In effect, engineering advances during the early 1930's produced planes that successfully withstood tremendous structural strains in the maneuvers of close combat or of dive-bombing, but the performance of their pilots was abruptly handicapped at crucial moments by gross disturbances of circulation, vision, and consciousness. The full military effectiveness of these planes was limited, therefore, not by technical but by physiological factors. It was the function first of the Committee on Aviation Medicine of the National Research Council, and then of the Subcommittee on Acceleration, to consider the practical aspects of the physiological problem and its possible solutions.

Devotion to a common cause, defeat of the enemy air forces as quickly as possible, led from the very beginning to complete pooling of theories, ideas, and evidence in this new field of physiology. The same co-operation was applied to methods of protection and to trials of protective devices under service conditions. In all respects the Committee on Aviation Medicine and the Subcommittee on Acceleration would have accomplished little but for the immeasurable advantage of sharing in the early problems and experiences of the military services in the United States, the British Isles, Canada, and Australia, as well as the pioneer researches of the Canadian Associate Committee on Aviation Medical Research and the Australian Flying Personnel Research Committee.

In retrospect, theories and methods were diverse enough to be stimulating and similar enough to establish continuity of effort. Two examples may be chosen from many to illustrate this point. In Canada, the development of a large human centrifuge, powered by a high-torque motor, was well advanced in 1940 and was in continuous operation early in 1941. A different principle and type of construction were used at Montreal for animals and in Australia for human beings. In the United States the Acceleration Laboratory of the Mayo Aeromedical Unit used still another principle, a rotating flywheel of large mass, to which the rotating arm and cockpits could be connected or disconnected at will by friction clutches. The advantages and disadvantages of each type of construction were compared at frequent intervals, and lessons from the common experience were incorporated in planning and constructing each of the later centrifuges at Wright Field, Pensacola, and the University of Southern California.

The same stimulating diversity of theory and practice held true also for protective devices. In 1939–1942 these included, in plans or in the early stages of investigation, the initial pneumatic abdominal support developed by Captain Poppen for the Navy, the hydraulic or water suit devised by the Royal Canadian Air Force, the gradient-pressure suit and gradient-pressure G-valve of the Navy, and the simplified single-pressure suit and arterial-occlusion suit of the Mayo Aeromedical Unit and Wright Field. To these must be added the aid and technical skill and advice provided by numerous manufacturers.

This review is necessarily too brief to do justice to any of these contributions. They are being covered much more adequately in a comprehensive monograph to be published shortly by the Subcommittee on Acceleration. In the present account, dealing as it must primarily with the work of the Subcommittee on Acceleration, only a fraction of the whole development can be covered, with a cursory summary of over-all accomplishment rather than a complete review of the ingenious experiments performed. In fact, in this field, as in others, the record of the war years is remarkable for its exploitation, elaboration, and application of existing knowledge, rather than

for the uncovering of any new fundamental information. The emphasis on quick practical results has left the fundamental physiology of acceleration in almost the same state as in 1940. Even now it cannot be stated surely whether protective devices increase tolerance of G [1] by increasing venous return, by diminishing the peripheral arteriolar bed and thereby increasing peripheral resistance, or, as seems more likely, by a combination of both mechanisms. It is definitely known, however, that extremely valuable protection against forces between 1 and 2 G can be provided for combat pilots by several relatively comfortable methods, and that this protection can be measured safely and objectively in centrifuges and planes.

Work on acceleration in the United States was necessarily limited to a few groups, partly because of difficulties in procurement of all instruments during wartime, and partly because each complete unit required a large human centrifuge with intricate control and recording mechanisms, a great number of subjects, and preferably easy access to combat planes of advanced design. Of the six civilian groups participating in studies on acceleration only two, the Aeromedical Unit of the Mayo Clinic and the Aeromedical Laboratories of the University of Southern California, had human centrifuges. A small centrifuge for animals was located at the University of Virginia. The remaining groups used civilian or service centrifuges as needed, or limited their work to laboratory studies.

As mentioned above, uniform motion or speed *per se* is harmless to the human being, provided wind blast is avoided by enclosing the pilot in a protective cockpit. Whether this will also be true of still higher speeds, such as may be developed in jet propulsion, remains to be seen. Certainly the speeds already attained have produced no deleterious effects, and it may be surmised that speed as such will not be a serious problem in aviation medicine, particularly when it is recalled that objects on the earth's surface are in constant motion at a rate of over 1000 miles per hour, or about 18.5 miles per second, due to the earth's rotation.

In practice, however, movement at strictly constant velocity, termed "uniform motion," is seldom observed; nonuniform motion or changing velocity is the rule, particularly in the plane during flight. Change in velocity per unit time is termed "acceleration." During flight this acceleration may take three forms: angular, radial, and linear. Each of these accelerations may be positive or negative in sign.

ANGULAR ACCELERATION

Angular acceleration occurs when the plane rotates around its long axis, as in a tight spin. The effects produced on the pilot can be compared to

[3] This term is explained on page 235.

those experienced during and immediately after rapid rotation in a Barany chair. Vestibular stimulation produces vertigo, nystagmus, forced movements, muscular inco-ordination, disorientation, and nausea and vomiting. Evidence for important circulatory effects is very fragmentary, although reduced blood pressure secondary to nausea has been described. In any case, angular acceleration can be a serious complication when a plane falls to earth out of control. It is a relatively unimportant, although disagreeable, factor in controlled flight or combat maneuvers. The subjective effects of this type of acceleration have also been experienced in mild degree by most subjects during rotation in human centrifuges, particularly in the earlier ones of small radius. As the design of centrifuges progressed to a larger radius and to improved cockpits, the effects became negligible except in a few extremely susceptible persons. In short, the effects of angular acceleration are of minor significance compared to the two stress-producing accelerations, radial and linear.

RADIAL ACCELERATION

Radial acceleration appears whenever the path of flight deviates from a straight line, as in a turn within a small radius, a diving spiral, or especially a pull-up from a dive at high speed. With this change in the direction of flight, the lift of the wings produces a centripetal force at right angles to the long axis of the airplane. Since the pilot is seated with the long axis of the body at right angles to the plane of the wings and to the line of flight, inertia presses his body into the seat of the plane by a centrifugal force of exactly the same magnitude. This type of acceleration has therefore been variously termed radial, centripetal, or centrifugal; the first term is generally preferred.

The magnitude of this force is related inversely to the radius of the curved path of flight and directly to the square of the plane's speed, in accordance with the equation $F = \dfrac{mv^2}{r}$, in which F is the force, v the velocity, m the mass, and r the radius of the curved path. Since weight constitutes a measure of the force of gravity and is proportional to the mass of a body, it is convenient and customary to express the force produced by radial acceleration in multiples of the earth's gravitational force. An airplane and pilot in level flight at constant velocity are influenced only by the normal gravitational force of the earth, or 1 g, but in a turn both are acted on also by the additional force due to radial acceleration; the latter is designated by the symbol G. The net result on plane and pilot is the vector sum of the two, because the effect of the earth's gravitational force depends at any moment on the orientation of the plane with reference to the earth's surface.

The force G, due to radial acceleration, can be calculated from the equation $F_{accel.}$ (or G) $= \dfrac{v^2}{32.2r}$, in which v is velocity in feet per second and r is the radius of the turn in feet. By common usage radial accelerations acting on the body of the pilot in the direction of head to feet are called positive ($+G$), and those acting in the direction of feet to head are called negative ($-G$).

Thus, a plane moving at a velocity of 200 miles per hour in a horizontal turn having a radius of ¼ mile exerts on the body of the pilot (whose head is toward the center) a force of 2 G. The pilot's weight of say 180 pounds with normal gravitational force then becomes 360 pounds at 2 G, and all the body tissues and fluids remain correspondingly heavy as long as the horizontal turn lasts. At 400 miles per hour, with the same radius of ¼ mile, 8.1 G will be developed and the pilot will then press on the seat with a weight of 1458 pounds. The effective specific gravity of his blood will be well over that of mercury, and the hydrostatic pressure of the blood in the region of the ankles of an average-sized pilot in the conventional seated position will be over 650 mm. of mercury, plus the existing arterial pressure due to the action of the heart. This totals about 15 pounds per square inch, compared to the normal of about 2 pounds per square inch.

LINEAR ACCELERATION

Linear acceleration results from changes in velocity of movement in a straight path. With the pilot in the present conventional sitting position, these forces act transversely across the body at right angles to its frontal plane; that is, from back to front or from front to back. Transverse G is met with to a very minor degree in all takeoffs and braked landings. In catapult takeoffs and in specially braked carrier landings transverse accelerations of 2, 3, or rarely 4 G may occur, but these have not so far offered any significant physiological problem, first because of their very brief durations at peak G, usually 0.5 to 1.5 seconds, and second because, as will be shown later, the human body is affected very little by G directed at right angles to the large blood vessels, although it is extremely vulnerable to similar G forces acting parallel to the larger arteries and veins.

Abrupt deceleration, as in crash landings, may of course produce a force of several hundred G, which, although acting for only a few milliseconds, causes fatal structural damage. Linear decelerations of important magnitude and duration also occur during the opening of parachutes, but work on these and related subjects was not considered in detail by the Subcommittee on Acceleration. It is highly probable that protection against linear acceleration will become more important in the future with reference to the pick-up of grounded personnel by planes in flight, the higher velocities of jet planes,

more rapid catapulting, and the expulsion of pilots from damaged planes by explosive charges. In the recent war, however, these innovations were just becoming challenging problems, and work on them will not be described here.

EFFECTS OF POSITIVE RADIAL ACCELERATION; MEASUREMENTS OF G TOLERANCE

The subjective sensations occurring during positive radial acceleration were well known by 1940 from published descriptions by pilots and from earlier observations in centrifuges. The first impression derived from the impact of aircraft against the resistance of the air during a pull-up from a dive is one of bodily strain. The cranium feels heavy, and as acceleration increases it becomes more and more difficult to hold the head erect. The skin of the forehead, eyelids, and cheeks sags and wrinkles; the eyeballs are driven downward in their sockets, and the lower jaw sags. The arms and legs feel heavier than normal, in proportion to the G developed, and they can be moved only with great effort. The lower legs feel congested and tense; cramps or tingling may occur. Expiration becomes difficult as the diaphragm is pulled down and the anterior abdominal wall is pushed out by the increased weight of the liver and other viscera. Depending somewhat on tolerance, but usually at between 3 and 4 G in the relaxed subject, visual disturbances begin to appear. Dimming or graying of peripheral fields occurs first and is followed rapidly at 4 to 5 G by total loss of vision, amaurosis fugax or "blackout." During this time consciousness persists and the pilot, warned by visual symptoms, can avoid further trouble by reducing the sharpness of the turn. With continued or still higher stress — for example, 5 to 6 G — consciousness is abruptly lost. Motion-picture studies and direct observations in centrifuges have shown that this unconsciousness is sometimes associated with mild or severe convulsive movements.

Subsidence of G is followed first by return of consciousness, associated with transient mental confusion and brief amnesia. Vision returns to normal within a few seconds, and there are no remote aftereffects except for a feeling of fatigue or lassitude, which may persist for some hours if many episodes of blackout, and particularly unconsciousness, have occurred.

Perusal of the literature published up to 1940 made it evident that, good though many descriptions might be, they were not quantitative enough to permit assaying accurately the exact protection afforded by protective suits. Objective measurements had been made in animals, however. Direct recordings by cannula and special manometer had demonstrated the conspicuous reduction of arterial blood pressure in dogs during acceleration, and similar results had been published from Holland. These methods were not suited to routine tests of subjects in centrifuges or of pilots in planes.

The Germans from 1933 on became obviously and increasingly interested in the physiology of blackout in man and, be it noted, published their findings openly in various journals at least until 1940. They identified during radial acceleration increasing tachycardia, accumulation of as much as 700 cc. of blood in the lower extremities, failure of venous return to the heart in man and monkeys as shown by x-ray studies of the cardiac shadow, respiratory embarrassment, important individual variations in tolerance, and the relation of symptoms to the grade, and also the duration, of G. Observations on blood pressure in man were few, poorly executed, and not in agreement. As a whole these German studies were badly controlled and not convincing. Although several ingenious methods of protection were studied superficially, they were not carried to the stage of practical usefulness except for recommending that the pilot use step-up rudder bars and assume a crouching position during G. In fact, in some instances the performance of superior planes was apparently limited purposely to avoid blackout of the pilot.

At the time of the first meetings of the Committee on Aviation Medicine and the Subcommittee on Acceleration, it was evident that the mechanical distention of the blood vessels below the heart, the failure of venous return, and the falling arterial blood pressure above the heart were fundamentally responsible for the symptoms described. It was equally evident that studies of the physiological responses to acceleration, particularly in man, had been chiefly hampered by the lack of objective methods for recording circulatory changes during flight. The dependability of subjective impressions, as related by pilots after flights, varied from subject to subject. They were often affected by the mental confusion associated with severe blackout and unconsciousness. For the purpose of exploring the possibility of selecting personnel maximally resistant to G, and particularly for assaying the value of protective measures, it seemed essential to obtain objective records of as many circulatory events as possible.

In Canada the possibility of selecting personnel on the basis of their resistance to G had already been under study by means of recording heart rates, blood pressure, and electroencephalograms in subjects on the tilt table and in the centrifuge. From these preliminary studies it appeared that if selection could be used at all, there was no substitute for actually testing each subject for his tolerance to G in the centrifuge. The need for several human centrifuges in the United States seemed clear.

With the advice and encouragement of Captain Poppen, initial efforts in this country were directed toward the development of methods for the objective and quantitative measurement of certain key circulatory changes by means of portable equipment suitable for use in the plane or by means of the centrifuge. Of these key changes, the amount of blood reaching the head, the heart rate, and the amplitude of pulsation in the temporal artery seemed most likely to yield immediately practical information.

For the plane particularly, and to a lesser degree for the centrifuge also, the standard laboratory methods of the circulatory physiologist were inadequate because of the conditions associated with dive-bombing maneuvers; *viz.*, rapidly changing atmospheric pressure, temperature, gravitational force, and vibration. Rapidity in recording was also essential because symptoms appear and disappear during G within 5 to 12 seconds. One of the useful methods developed was the photoelectric measurement of so-called "ear opacity" to indicate rapid changes in cephalic blood content. This method was first designed for use in planes, but was quickly adopted in all centrifuge installations and employed for determining tolerance to G, with and without protection, in large numbers of subjects.

Changes in ear opacity were measured by means of the lamp and photoelectric cell in the earpiece of a Millikan oximeter, connected through a suitable amplifier and rectifier to a cathode-ray oscillograph (for the plane) or to a string galvanometer (for the centrifuge). The amount of light transmitted to the photoelectric cell by the tissues of the ear depends on the relative opacity of the bloodless stroma of the ear, comprising about 70 to 80 per cent of total opacity, and the relative opacity of the blood within the blood vessels. The latter depends first on the total amount of blood per unit of tissue and second, as shown by Millikan, on its oxygen saturation. Other factors remaining constant, a reduction in total opacity of the ear can be used to indicate reduction in the blood content of the ear tissues and thereby reduction in blood supply to the brain and head as a whole. A convex glass can also be inserted between the lamp and ear so that, for calibration, the tissues of the ear immediately between the photoelectric cell and the lamp can be compressed until completely blanched. Comparison of the opacities measured during compression (that is, in a bloodless state), during full circulation, and during the experimental procedure permits one to read the approximate percentage decrease or increase of blood content from calibration curves empirically determined for each ear unit with known opacities.

Figure 26 shows the photoelectric unit (modified earpiece of the oximeter, marked P) in position on the ear, and adjacent to it a Brush CH–1 quartz crystal microphone (marked M), used to record the amplitude of pulsation in the temporal artery. Figure 27 shows the compact recording unit for use in the plane. This unit contained a camera for 35-mm. film, two cathode-ray oscillographs (circuit 1 with amplifier and rectifier for the photoelectric unit and ear opacity, and circuit 2 with a straight-capacity coupled amplifier for the quartz crystal and pulsation of the temporal artery), horizontal and vertical accelerometers, and a signal light with which the subject could record symptoms by prearranged code.

In lieu of detailed description a few illustrative control experiments performed in the laboratory at the University of Virginia are reproduced in the upper part of Figure 28 (A and B). In a seated subject, compressing the

carotid artery obliterated pulsation of the temporal artery (lower line of A) and promptly reduced ear opacity (upper line of A) on the same side. Some subjects complained of unilateral dimness of vision on the affected side. A similarly close relation between blood content of the ear tissues and dimness of vision was observed in subjects on the tilt table just before syncope occurred. A Valsalva maneuver, on the contrary, increased the blood content of the ear, as would be expected from passive congestion (B).

Preliminary observations in a chartered Waco plane in 1942 (Army and Navy planes being then unavailable for experimental purposes) showed that the method was extremely sensitive, because it demonstrated reductions in ear opacity at 2.2 G, at which point visual symptoms had not yet appeared. By courtesy of the Toronto Aeromedical Unit, tests were carried out in the Toronto centrifuge in September, 1942. Figure 28 C shows (from above downward and at slower film speed) records of ear opacity, horizontal accelerometer, vertical accelerometer, and pulsation of the temporal artery. At 2 G ear opacity decreased conspicuously but the associated reduction of cephalic blood supply was not enough to produce visual symptoms. At 4 G (Fig. 28 D) a greater decrease in blood content was associated at first with dimming of vision and finally with blackout. Ear opacity began to return toward normal before vision returned. In this same subject 5 G produced even greater reduction of ear opacity, associated with unconsciousness. Repeated runs with the same subject provided remarkably consistent records under standard conditions and permitted objective measurement of the protection afforded by posture or by suits during standard tests in centrifuges and later in planes.

Vibration did not affect the records of ear opacity but interfered with the recording of arterial pulsation by quartz crystal (Fig. 28 C and D). The tachycardia and obliteration of pulse in the temporal artery, characteristically found in blackout, are merely suggested in the irregularities of the lower record of D. Changes in pulsation were recorded by the Mayo Aeromedical Unit more successfully by amplifying the minute pulse of the ear opacity curve (seen best in A and C) to record so-called "ear pulse." This has proved to be another sensitive indicator of cephalic ischemia and shows changes in some situations more rapidly than does ear opacity itself.

The developments in the simultaneous recording of numerous physiological events during acceleration can be shown best by comparing Figures 29 and 30. Figure 29 has been reproduced from Captain Poppen's report because it represents one of the very first records demonstrating the fundamental cause of symptoms during radial acceleration. In a plane with a dog in the head-up position, to simulate the position of the pilot, 4 G produced a profound fall of arterial blood pressure, which had not yet reached its lowest point when the maneuver ceased 8 seconds after starting. The upper curve

shows the smoked-paper tracing produced by the specially constructed G-proof manometer; the lower curve, an analysis of an original tracing, indicates the rapidity with which blood pressure falls and recovers. Figure 30 shows the record of a recent observation on a human being in the Mayo centrifuge. In order to register the many concurrent reactions to G, recording was expanded to include instantaneous records of heart rate, electrocardiogram, ear pulse, blood content of the ear, arterial blood pressure at head level, actual G at head level, and the subject's responses to the flashing on of peripheral lights and central lights. Peripheral vision was tested repeatedly by snapping on two lights, each 30 inches from the eyes and at an angle of 23 degrees to a line drawn forward from the bridge of the nose. The subject, by means of switches on the stick in the cockpit, snapped these lights off immediately; failures of peripheral vision and central vision were indicated separately by his failure to respond. The length of the upper lines in each instance permitted computing visual reaction times under these conditions.

In Figure 30, for instance, 4 G for 15 seconds produced obvious tachycardia, a transient decrease of ear pulse, a pronounced decrease of ear opacity, profound reduction of arterial blood pressure at head level, and failure of peripheral vision with retention of central vision. At about 9 seconds after onset of maximum G the carotid sinus or moderator mechanism, stimulated by hypotension, produced vasoconstriction and an elevated arterial pressure, increased the blood content of the ear, and restored peripheral vision, despite continued G. Exposures to the given G for periods shorter than 10 to 15 seconds may obscure this recovery phase or confuse it with the effects of withdrawing G. Moreover, in some instances short exposures do not demonstrate the full symptomatic effect of a given grade of G because very brief exposures to high G — for example, 6 G for 1 second — do not affect blood pressure significantly, presumably because of inertia and consequent delay in reaching the new equilibrium. The great advantage of multiple recordings is obvious: they permit comparing tolerance to G and determining effective protection by suits, in terms of numerous objective criteria in addition to subjective impressions.

The concurrent developments in centrifuge construction and instrumentation can also be illustrated by photographs. Figure 31 shows the first human centrifuge in this country, built during 1936 at Wright Field. Figure 32 shows a view of the central pillar and one half of the rotating arm of the Mayo centrifuge. The swivel-mounted cockpit with subject is shown to the left, the central pillar and observer's seat with controls at the extreme right. During rotation the cockpit swings outward and the subject's heart is 15.5 feet from the axis of rotation.

Motion-picture recording of facial expressions added further details (Fig. 33). The portable oscillographic unit developed at the University of Virginia

(see Figs. 26, 27, and 28) was placed in a plane by Dr. Lambert of the Mayo Aeromedical Unit, with the addition of a motion-picture camera. The transition from 1 to 5 G was marked by sagging of the loose tissues of the face, reduction of blood content of the ear, disappearance of ear pulse, blackout, and semiconsciousness, followed by a period of disorientation, which persisted several seconds after return to 1 G. It is this brief period of twilight consciousness that makes excessive G hazardous and renders subjective impressions of questionable value.

To describe adequately the factual and quantitative information yielded by these and other methods is impossible in a few pages; only a brief summary will be attempted. The extensive studies of the Mayo unit have demonstrated two distinct periods in the reaction to positive radial G lasting for 15 or more seconds. The first period, that of progressive failure, is characterized by mounting tachycardia, decline of arterial blood pressure at head level, increasing facial pallor, reduced cephalic blood supply as indicated by ear opacity, reduction and disappearance of ear pulse, and finally successive loss of peripheral vision, central vision, and consciousness. The full measure of these changes is reached in 6 to 11 seconds after onset of maximum G. It is interesting in comparison that other workers observed unconsciousness within an average of 5 to 6 seconds after the carotid and vertebral arteries were completely occluded by suddenly inflating a cervical pneumatic cuff.

The second stage, that of compensation, is characterized by slight decrease, or at least arrest, of tachycardia, a slight rise of blood pressure at head level, an improved cephalic blood supply as indicated by ear opacity, return of ear pulse, and, if this improvement is sufficient, recovery of consciousness and vision. At moderate G this compensation may produce sustained improvement for long periods except for occasional wavelike lapses. At high G, however, compensation is limited because the moderator mechanism is overwhelmed and ineffective.

Subsidence of G is accompanied by a pronounced reactive hyperemia in the cephalic region, as indicated in color photographs by vivid flushing of the facial skin. This is associated with return of all the criteria to normal or, briefly, slightly above normal. The importance of the moderator mechanism is indicated by several observations. First, compensation of one short exposure to G protects the subject in later exposures, provided the interval between successive exposures is not longer than 10 seconds, as shown at Wright Field. Second, positive G following immediately after even minor negative G produces particularly severe blackout at less than normal G thresholds, presumably because the moderator mechanism has been stimulated to full depressor action by the rise of carotid pressure produced by negative G. Then, caught, so to speak, unawares, the immediately subsequent positive G is not resisted even by normal vasomotor tone. Third, in animals the reduction of arterial pressure with given G is greater after severing of the moderator

nerves; there is no compensatory rise in blood pressure and no overshoot afterward.

It was not known originally how near these striking functional changes might be to permanent organic injury or death. Actually the margin of safety is very wide, as indicated by the lethal limits of positive G in several species, including monkeys. To kill over 75 per cent of a group of rats required 25 G sustained for 2 minutes. In man 6 G for 10 seconds produces unconsciousness in all but a few instances. Taking intensity and duration of stress into account, the exposure to G that produces disabling symptoms is at most one tenth, and probably nearer one fiftieth, the exposure that is lethal. This finding, together with accumulated experience with men carried to blackout in centrifuges, provided greater sense of security in repeated testing such as is required in measuring the effectiveness of protective measures. Nevertheless, repeated unconsciousness has been avoided in centrifuge tests because of the fatigue it produces and the unknown effects of many episodes of profound cerebral ischemia. No organic damage has been reported for young and vigorous subjects, although they have been blacked out many times. It has been claimed that in planes high G can sprain or even fracture the spine when the body is bent forward in the crouching position recommended by the Germans, but nothing of this sort has been seen in the centrifuge. Electroencephalographic changes appear frequently only with unconsciousness; slow waves of large amplitude are then found but, according to the Canadians, are not uniform enough in their appearance to be helpful in pilot selection. In general, primates resist G and compensate better than do lower animals, presumably because they are already conditioned by continuous exposure to 1 G acting in their long axis, from head to foot. Excised human arteries and veins withstand, without rupture, internal pressures about twice those produced by 9 G. Inconsequential, minute petechial hemorrhages are sometimes seen in the lower extremities, but these are due to rupture of small venules or capillaries.

The reason for the extinction of vision before consciousness is now known to be clearly referable to the local handicap that an intraocular pressure of 20 mm. of mercury places on the retinal circulation. The Mayo unit showed that suction cups, reducing pressure on the eyes to −30 mm. (that is, below atmospheric pressure), neutralized intraocular pressure and raised blackout threshold to coincide with that for unconsciousness. Conversely, pressure on the eyes made vision more than usually vulnerable to G but left the threshold for unconsciousness unaffected. At 1 G — that is, under normal conditions — an external pressure of 65 mm. compressed the retinal arterioles and produced blackout.

Conditions inside the cranium offer an interesting contrast. In animals with transparent windows in the cranial vault positive G did not render the brain bloodless but merely stopped flow completely. The vessels could not

collapse, because as G reduced arterial pressure it concurrently made cerebrospinal pressure negative. The blood in these vessels became blue because flow had ceased, but the vessels did not empty.

Important pressure relationships in another body cavity were studied in animals and man at Randolph Field. Normally at 1 G intra-abdominal pressure, measured by an intrarectal balloon, was great enough to support a column of blood from any level in the abdomen to within an average of 5.4 cm. below the diaphragm. During positive G in the centrifuge, intra-abdominal pressure increased proportionally with G, but not enough to match the levels that would be predicted on the basis of a column of fluid of constant height. This discrepancy was ascribed to protrusion of the anterior abdominal wall and compression of intestinal gas by the weight of the viscera above. Measurements suggested that the diaphragm and heart descended 7.2 cm. at 5 G, thus increasing the normal heart-to-head distance and adding a further mechanical handicap calculated to be approximately 0.5 G.

In detailed measurements of arterial blood pressure in man the Mayo unit showed that systolic blood pressure at head level decreased within 7 seconds after onset of G by 20 to 36 mm. of mercury (average 28 mm.) per G above 1 G. At heart level the decrease was only 6 mm. per G. Compensation by the moderator mechanism raised blood pressure at head level on the average 35 mm. above the lowest level reached during G, and produced at heart level total pressures of 150 to 170 mm. Ear opacity and blood pressure changed together in closely correlated fashion, whereas tachycardia changed inversely to both.

Tolerance varies considerably from subject to subject and is much modified by the completeness of muscular relaxation. In Toronto the blackout threshold was found to range from 2.5 to 9 G, the mean being 5 G. These studies were made with duration of maximum G limited to 5 seconds, to simulate stresses apt to be met in pursuit craft. In a group of subjects 100 per cent tolerated 2 G without blackout, 80 per cent tolerated 4 G, 20 per cent tolerated 6 G, and about 2 per cent tolerated 8 G. The German concept that resistance to G depends on heart-to-head distance and general body build was found to be erroneous.

Individual variation was greater in this series than in that studied by the Mayo group, where exposures of 15 seconds were used routinely, with great pains taken to obtain complete muscular relaxation throughout each test. Both types of observation are helpful for special purposes, and there seems to be little point in arguing which procedure is better. In testing suits relaxation and longer exposure are preferable to avoid error; the recorded protection may be somewhat less than would be observed in combat, where shorter exposures to G are the rule, but comparisons between various suits are bound to be more accurate if the factors of relaxation and duration of

stress are excluded. For this reason in the United States exposures of 10 to 15 seconds have been used almost routinely. The tolerances given above apply, of course, to subjects in the conventional sitting position of the average pilot's seat. In the full standing position blackout and unconsciousness have been observed at levels as low as 2 and 2.5 G.

Under controlled conditions diurnal variation of a given trained subject is usually within 0.4 G or less, and variations are not regular from hour to hour. A warm environment, 89° F. and 77 per cent humidity, reduces tolerance to 1 G less than that found in a cold environment, 43° F. and 72 per cent humidity. Minor infections, gastroenteritis, and excessive fatigue lower the threshold. The University of Southern California unit found that hypoglycemia and hyperglycemia respectively reduced and increased the threshold, according to several criteria, by an average of 0.2 G. A stomach filled with water, milk, or food increased tolerance by an average of 0.4 G, rather than 0.9 G as claimed by the Germans. This protection seems to be mechanical in origin, since water and food act similarly. Measurements of intra-abdominal pressure support this explanation, because a full stomach magnified the increase of intra-abdominal pressure produced by given G and thereby improved the external support of the large and volumetrically important splanchnic vascular bed.

The Southern California unit also studied the loss of fluid from the vascular system due to filtration by the high capillary blood pressure in the dependent parts of the body during G. Fluid loss after 5 minutes at 3.5 to 5 G totaled 216 to 270 cc. Exposure to similar G for several short periods totaling 5 minutes, but with short rests between exposures, demonstrated rapid reabsorption of this fluid and excluded the possibility that reduced plasma volume was a serious hazard in many brief exposures.

Finally, although uniform speed *per se* produces no detectable physiological effects, it does have an important relation to the distance traversed in a single visual or auditory reaction time. Two planes traveling toward each other at 5 miles per minute, or 300 miles per hour, have a total speed relative to each other of 10 miles per minute, or almost 1000 feet per second. This speed is over twice the maximum rate at which the nerve impulse travels over mammalian nerve fibers. In a single visual reaction time (for example, 0.3 seconds under centrifuge conditions) the two planes will have drawn nearer to each other by 300 feet. The Toronto unit found, however, no systematic increase in visual reaction times at G below the blackout level, although all subjects showed a tendency to be inattentive in that they failed occasionally at low or high G to respond to some stimuli for an excessively long time. This inattention was observed more frequently as G approached blackout levels, although the general delay in reaction was not significantly greater. At blackout level, of course, all response to visual stimuli was in abeyance. Auditory reaction times were not significantly increased over the

normal at levels of G that produced blackout and a significantly longer time of response to visual stimuli. A protective suit not only preserved efficient visual responses at higher G, but also lessened the lapses due to inattention at all levels of G.

DEVICES FOR AMELIORATING THE EFFECTS OF POSITIVE RADIAL ACCELERATION

Viewed from the standpoint of physiology alone, the simplest and most logical method of ameliorating or avoiding the effects of positive radial acceleration is to place the pilot in the supine or prone position with the long axis of his body parallel or tangential to the line of flight. By 1937 it was known that men could withstand between 12 and 16 G without loss of vision or consciousness, provided the force of acceleration was directed transversely across the body. It is the conventional sitting position of the pilot that makes him particularly vulnerable to radial acceleration, because in this position the force of acceleration runs parallel to the larger arteries and veins.

The urgencies and exigencies of war, however, do not permit last-minute radical changes in equipment so complicated as the modern combat plane. The controls, armament, maneuverability, and visible horizon of these planes are planned on the basis of a conventionally seated pilot. To change the pilot's posture radically would mean rebuilding the whole plane. The construction of planes to be handled by prone or supine pilots, while seriously considered, never progressed beyond the earliest experimental stages. Had such planes been practical a whole new set of military tactics would have been available, and in fact required, because the prone or supine pilot could have withstood negative as well as positive radial acceleration. He could have used inside or outside turns with almost equal facility. With the best anti-G equipment now available he is somewhat protected against positive G, but is still as vulnerable as ever to "red-out" and the harassing aftereffects of -2 or -3 G; that is, acceleration directed from feet to head, as seen in outside turns with the head away from the center of the turn.

Anti-G equipment now available can raise the blackout threshold of the average pilot by approximately 1.5 G when exposure to G is prolonged to 15 seconds, and probably slightly more than that when it is reduced to 5 seconds, as is usually the case in close combat. At first glance this protection seems trifling, but its significance becomes more apparent when translated into terms of potential safe reduction of radius of turn at a given velocity and potential safe increase in velocity with a given radius of turn.

Let us suppose that a pilot and his adversary both maintain clear vision consistently up to 5 G, but not beyond. Without protection both can fly with full vision, for example, at a rate of 300 miles per hour in an arc hav-

ing a radius of 1200 feet. At 300 miles per hour neither can reduce this radius and maintain clear vision. Holding to a radius of 1200 feet neither can go faster than 300 miles per hour and maintain clear vision. If one pilot is protected to the extent of 1.5 G, he can then maintain clear vision during accelerations up to 6.5 G, and he has two possible advantages over his unprotected opponent. Maintaining a radius of 1200 feet he can fly with vision up to 340 miles per hour, whereas his opponent is limited to 300 miles. Or at a velocity of 300 miles per hour he can safely turn inside his opponent's path to follow an arc with a radius of 925 feet, whereas the opponent cannot reduce his arc of flight to less than 1200 feet without sacrificing vision. A 13 per cent increase in speed or a 23 per cent decrease in radius of turn is very worthwhile, as pilots have demonstrated in the course of training by matched "dogfights" with and without protection. Service tests showed that pilots with combat experience were eager to have this protection of 1.5 G provided it was absolutely dependable, was not too uncomfortable in the ready room, in the scramble, or during flight, was not too hot in the tropics, and did not restrict the freedom of movement required for constant scanning of the horizon to detect enemy aircraft at the earliest possible moment.

Many methods of improving tolerance to positive radial acceleration were tested quantitatively in laboratories and centrifuges. A selected few were subjected to testing under service conditions, referred back to the laboratory for modification, and then, after further testing, adopted for use in combat. The methods of protection studied in the laboratory and centrifuge, described below, can be classified as follows:

1. Self-protection
    (a) Muscle tensing, grunting, shouting, bearing down (pulling against weighted stick)
    (b) Taking of meals
    (c) Special breathing to produce alternately high and low intrathoracic pressures
2. Postural protection
    (a) Prone or supine position
    (b) Crouch and step-up rudder bars
    (c) Tilting seat
3. Pharmacodynamic agents
4. Protection by suits applying external pressure to the abdomen, legs, or both
    (a) Pneumatic abdominal belt
    (b) Hydraulic suit (water immersion)
    (c) Gradient-pressure pneumatic suit with valves activated by G
    (d) Single-pressure pneumatic suit and single valve activated by G
    (e) Arterial-occlusion suit with valve activated by G (progressive arterial-occlusion suit)
    (f) Pneumatic lever suit and valve activated by G
    (g) Net suit

1. Self-Protection

Long before 1940 commercial test pilots and combat pilots had learned by precept and experience their approximate blackout threshold and the dangers inherent in radial acceleration. So far as possible they kept their maneuvers within limits that they knew to be safe. The statement of one experienced test pilot, interviewed in 1940, was particularly enlightening. In testing the tolerance of new planes for specified G (for example, 8 or 9 G) he accomplished these tests, to use his own words, "by bracing myself, yelling, and whipping her around fast." The desired peak G was reached but lasted only 2 or 3 seconds. This was long enough to test the structure of the plane but not long enough to develop the full circulatory effects of 8 or 9 G, which in 6 seconds or more would have produced unconsciousness.

Pilots have learned that tensing of muscles, sustained grunting, bearing down, and shouting lessens symptoms at given G. These maneuvers, if well timed and co-ordinated, can be very efficient and are probably responsible for the extremely high tolerance to G exhibited by a few subjects even without protection. It is, in fact, quite difficult for the untrained subject to remain relaxed during centrifugation or during a dive and pull-up in a plane. The instinctive and involuntary reaction is to become tense and to "brace oneself against the G."

These measures favor the return of venous blood to the heart by increasing tissue pressure in the legs and by compressing the venules and veins there, at the same time increasing intra-abdominal pressure and compressing the large veins in the splanchnic region. Moreover, it is known that vigorous tetanic contraction of skeletal muscle can compress widely dilated arterioles and minute vessels completely enough to stop arterial inflow and arrest reactive hyperemia, at least at normal pressures. Whether this applies also to the higher arteriolar pressures met with in acceleration is not known, but it seems likely that the total arteriolar bed in the extremities can be reduced significantly, at least for short periods, by sustained vigorous isometric contraction.

Opinion on the question of the mechanism by which protective devices, including suits, may act is still sharply divided. It is clear that the only way of achieving protection is to maintain a sufficient arterial pressure at head level at all times, but it is not known whether this protection is achieved by improving venous return or by reducing the outflow of arterial blood into regions other than the head. It seems logical to conclude that there is combined action of both mechanisms in varying proportions, rather than separate action by either. This theory is supported by the observed facts concerning self-protection and the effect of posture and suits. There are many reasons for believing that active self-protection and passive protection by suits im-

prove venous return but cannot restore it to normal even at moderate G. Under these conditions any diminution of the total cross-sectional area of the arteriolar bed, in the extremities or the abdomen, diminishes the outflow of arterial blood to these temporarily unimportant regions and conserves for the cephalic region a greater fraction of the already limited venous return. A combined mechanism of this sort explains the number and diversity of measures that improve tolerance for G, their additive effect when combined, and the inability of any single or combined procedure to improve tolerance of a seated pilot by more than 2.5 G at most.

Self-protective maneuvers depend so largely on individual effort that their quantitative effectiveness is hard to assess. The nearest approach to quantitative study was made in the Mayo centrifuge, where the effect of tensing the muscles was measured by comparing tolerance against graded G, first in the relaxed state and then while pulling against a stick weighted 19 pounds per G. The average protection, computed for several objective criteria, amounted to 0.7 G. If the stick was weighted only 10 pounds per G, protection was reduced to 0.3 G, which suggests that tensing of muscles must be extreme in order to be effective. It was also found that a pneumatic suit gave an average protection of 1.5 G to these same subjects when relaxed, but when this passive protection was reinforced by the active protection of pulling the heavily weighted stick, the combined effect was 2.1 G. The additive effects of passive and active measures seem clear.

The effect of a full stomach has already been mentioned. Tolerance of G is raised approximately 0.4 G, rather than the 0.9 G claimed by the Germans. This protection is apparently mechanical, because with a full stomach the rise of intra-abdominal pressure per G is greater than with an empty stomach and the support of the splanchnic veins is correspondingly greater.

Special breathing to produce alternately high and low intrathoracic pressures, as described by the Mayo group under the title of M–1 maneuver, can be extremely effective. Tolerance can be raised at least 2 G by a well-trained subject. The value of this maneuver was confirmed, and it was recommended as an emergency measure to compensate for failure of a protective suit.

In effect, the M–1 maneuver makes the thoracic cage an auxiliary pump, by exaggerating the shifts of intrathoracic pressure associated with inspiration and expiration. The directions are briefly as follows: pull in the chin, hunch the shoulders, push against the tightly drawn safety belt, and force air out of the lungs by prolonged grunting against a partially closed glottis. When all possible air has been expelled inhale deeply and rapidly and start again. Inspiration should last 1 second and expiration 3 or 4 seconds. Deep, rapid inspiration aids venous return, and forced expiration against resistance develops a pressure of 50 to 100 mm. of mercury. The average intra-abdominal pressure developed during acceleration in relaxed subjects was

about 25 mm. per G, but the maneuver just described could elevate intra-abdominal pressure to a total of 300 mm. during acceleration. Again, it may be noted, protection is associated with support of the splanchnic vascular bed.

2. POSTURAL PROTECTION

The reasons for delay in adopting the prone or supine position have already been discussed. The Germans went partway along this path, however, by supplying their planes with auxiliary, elevated step-up rudder bars for use when radial acceleration was necessary and by advising the crouch position during pull-outs. The crouch position consists of drawing the knees toward the abdomen, at the same time bending the trunk and head forward toward the dash. The high rudder bar diminishes pooling of blood in the legs by reducing the height of the hydrostatic column from heart to toes. The crouch compresses the abdomen, diminishes the hydrostatic column from heart to head, and reduces the arterial pressure required to lift blood to the head during G. It was claimed that crouching increased tolerance by 2 G, but 0.9 G seems a more accurate figure. The auxiliary pedals also give much less protection than claimed by the Germans. After early service trials these measures were abandoned because of reported fractures in the lumbo-dorsal region, coupled with complaints that in many planes the crouching position reduced the field of vision ahead and to the side of the plane.

Another approach to postural protection, a seat that tilted backward with increasing G, was studied in several centers. A 45-degree tilt gave protection of about 1 G, a 30-degree tilt only 0.5 G. Again the field of vision suffered. Provision for even minor tilting would probably require major changes in plane construction in some instances. The engineers habitually compress the pilot into the smallest possible space to save weight and gain speed. A tilt of slightly less than 90 degrees would, of course, provide the full supine position, which would yield maximal protection against both negative and positive radial acceleration.

3. PHARMACODYNAMIC AGENTS

The feasibility of using injections and medications prior to exposure to the forces of acceleration was never rated very highly because of the inability to foretell, at least in defensive warfare, just when a "scramble" would be necessary. Such measures would also have to overcome the natural distaste of normal, healthy persons for the artificial aid of medicines or, to use the pilot's term, "dope." This distaste might have been allayed by indoctrination if any really effective medication had been found, but this was not the case.

Captain Poppen in 1933 found that ephedrine did not reduce the decline

FIGURE 26. *Photoelectric unit (P) for measuring opacity and blood content of ear tissues.*

FIGURE 27. *Portable oscillographic unit for recording ear opacity, pulsation in temporal artery, heart rate, and horizontal and vertical acceleration.*

A. Effect of compressing common carotid artery, same side.

B. Effect of Valsalva maneuver

C. Effect of acceleration, + 2 G. maximum

D. Acceleration, + 4 G maximum

Greying   Blackout   Normal vision returned
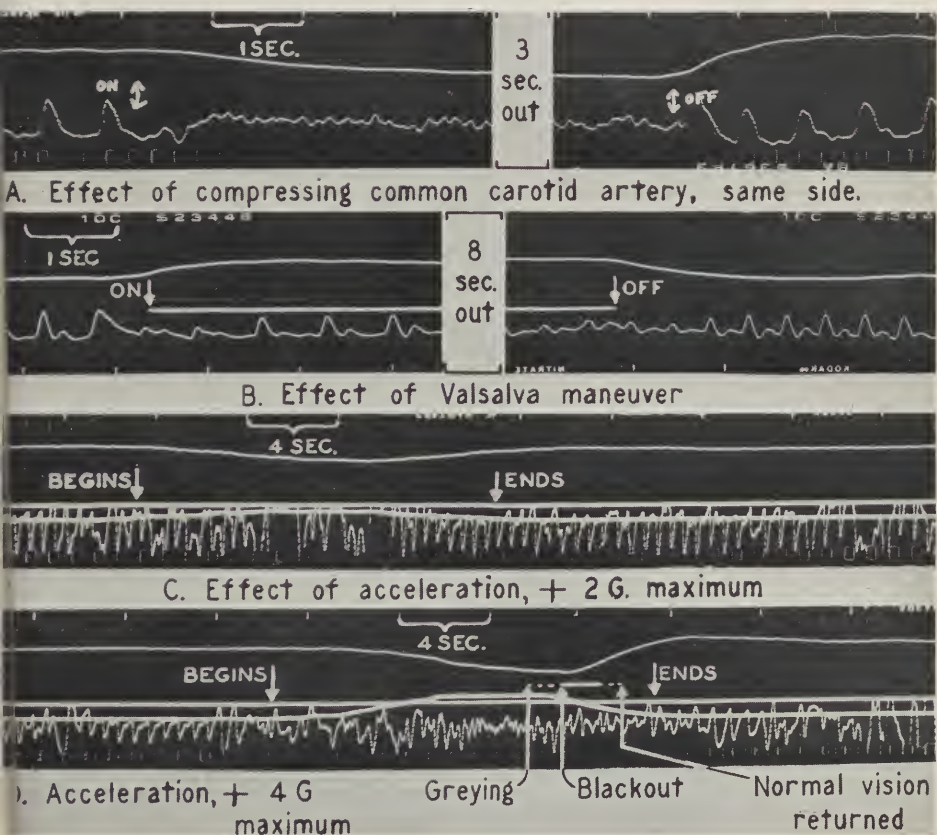
FIGURE 28. *Records of changes in the blood content of the ear during control experiments.*
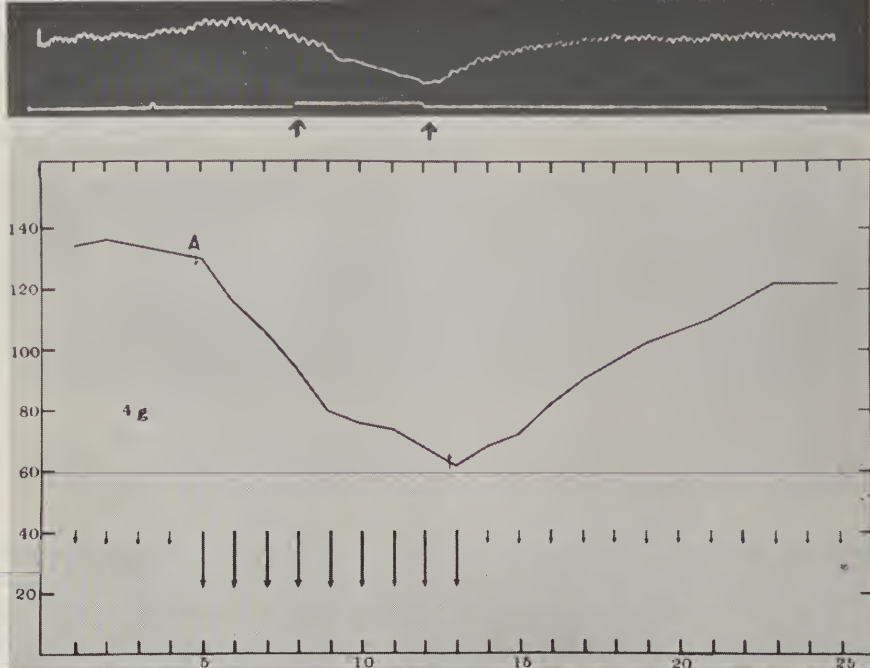
FIGURE 29. *Records showing decline of arterial pressure in a dog during exposure to positive radial acceleration.*
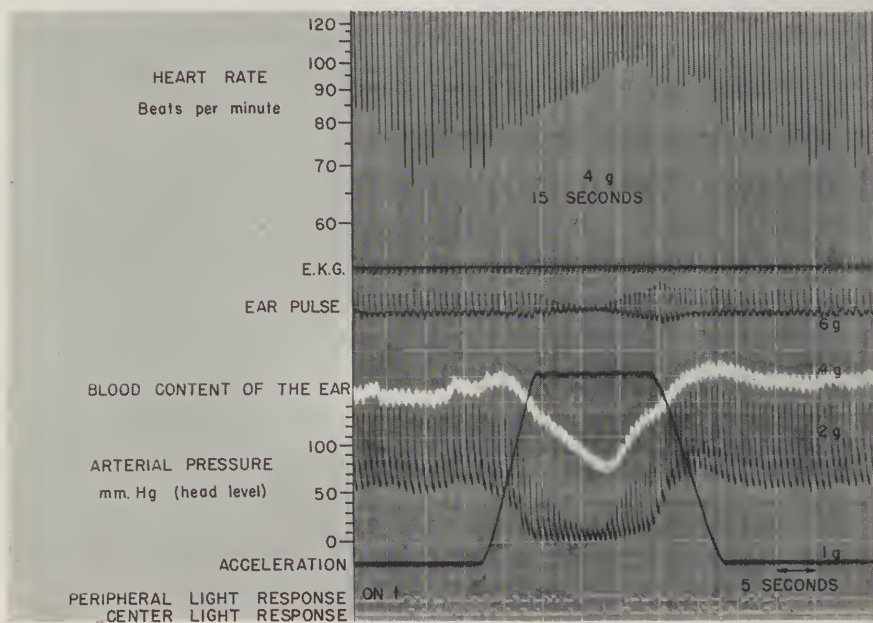
FIGURE 30. *Sequence of physiological events during exposure of a human being to 4 G for 15 seconds.*
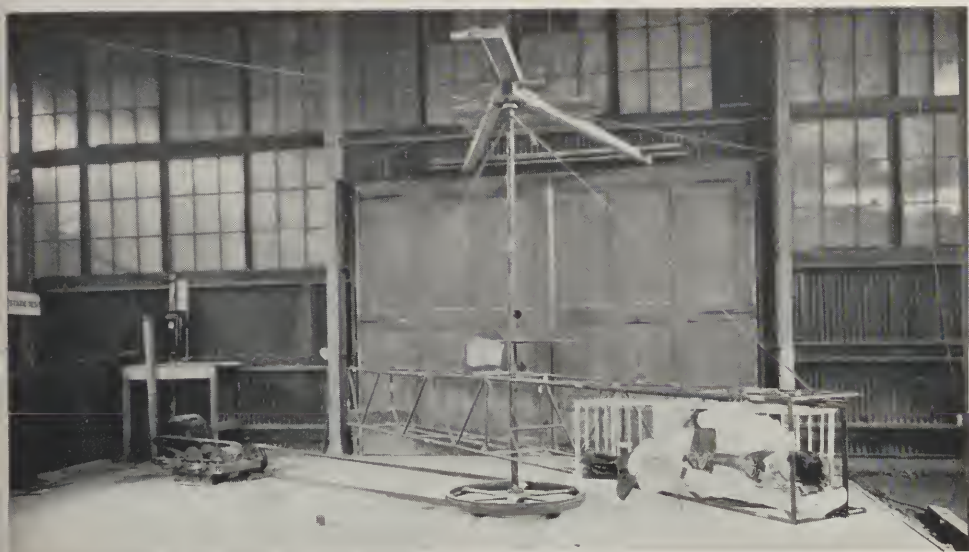
FIGURE 31. *The first human centrifuge in the United States, built in the Aeromedical Research Laboratory, Wright Field, by Colonel H. G. Armstrong and Dr. J. W. Heim.*
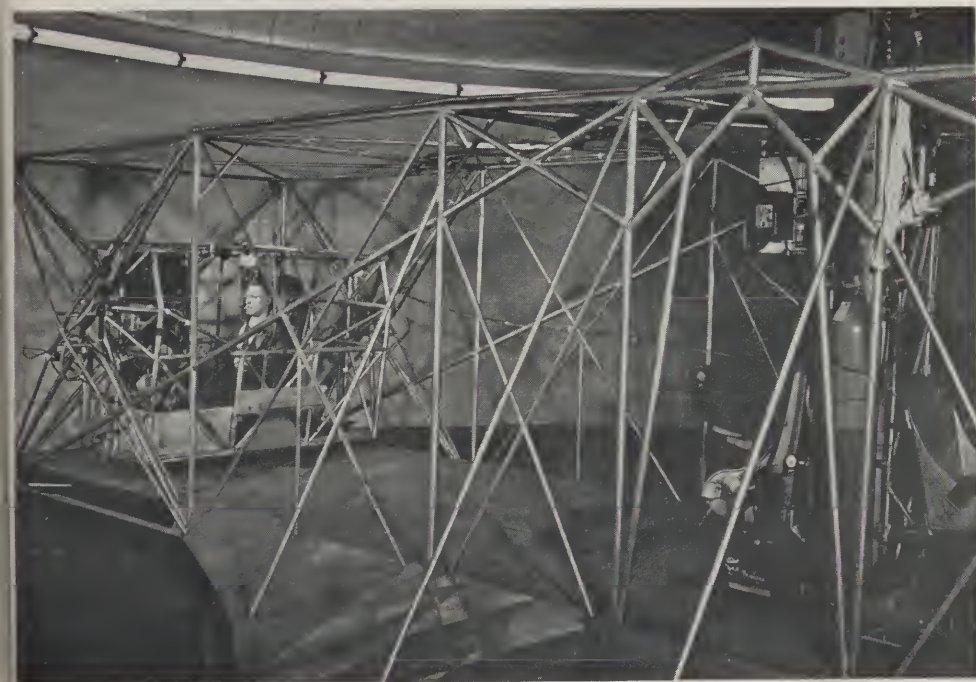
FIGURE 32. *Mayo centrifuge in the Acceleration Laboratory, Mayo Aeromedical Unit.*
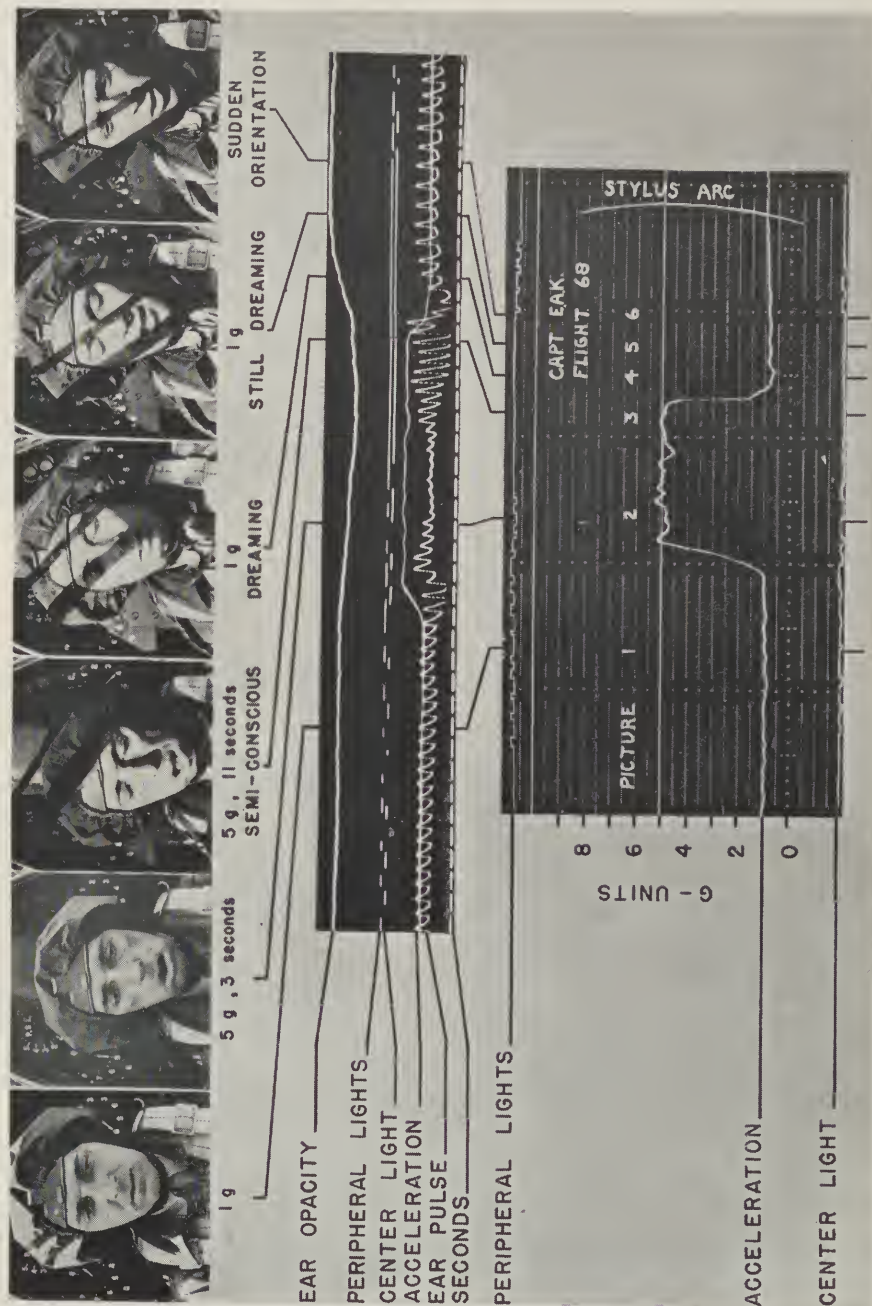
FIGURE 33. *The effect of +5 G acceleration on an unprotected passenger in an airplane.*

of blood pressure observed in dogs during dives in a plane. The Canadians detected no protective effect from the use of coramine, benzedrine, glucose, ephedrine, adrenalin, adrenocortical extract, desoxycorticosterone, or fraction. E. Britton et al. observed in animals some protection at very high, prolonged G by pitressin, pituitrin, exposure to cold, and desoxycorticosterone, but the doses were large and generally inapplicable to man for the reasons mentioned above. Both Jasper and Britton observed improved tolerance to G in animals made polycythemic by acclimatization to anoxia, but Jasper observed in these animals the serious disadvantage of delayed recovery. The Germans had described slight protection from inhaling 7 per cent carbon dioxide in oxygen; Britton found that in animals much higher concentrations were required for slight protection.

In summary, the results obtained with pharmacodynamic agents have not so far justified serious service trials in any instance.

4. Protective Belts and Suits

The first protective garment was described by Captain Poppen in 1934. It operated on the principle of supporting the vascular tree by external pressure. It was postulated that pooling of blood in the splanchnic region was the most important factor in the decline of arterial pressure that occurred in dogs during radial acceleration in planes. It was suggested that elevating the intra-abdominal pressure should correct this arterial hypotension, at least partially, by improving venous return. Captain Poppen demonstrated that a simple, tight binder was not effective, that an inflatable abdominal belt was necessary, and that inflation of such a belt should precede slightly the onset of G. With such belts he observed in dogs lessened reduction of arterial pressure during radial acceleration. Promising preliminary results were described in man, but Captain Poppen's transfer to other duties prevented further research in this direction.

In 1940 the effects in man of pneumatic belts, inflated to 100 mm. of mercury, were studied in more detail. The investigators observed partial protection against the impaired venous return and arterial hypotension of nitrite syncope on the tilt table. At about the same time service tests in planes indicated that abdominal support increased the threshold for blackout slightly, but that if this raised threshold was exceeded the ensuing blackout was likely to be more sudden, more severe, and probably more prolonged. This was attributed to the trapping of blood in the veins of the legs below the belt and supported the logic of the next step — the simultaneous compression of the legs and abdomen.

This type of support was studied in several different forms. In Canada the Franks hydraulic suit received most study, in Australia Cotton's gradient-pressure pneumatic suit, and in the United States first a gradient-pressure

pneumatic suit of Navy design and then a simplified single-pressure suit and its variants.

The basic purpose of all these garments was the same; *viz.,* to improve venous return, to maintain so far as possible a normal arterial blood pressure at head level, and to improve the cephalic blood supply. This was accomplished by applying to the abdomen and legs external pressure sufficient to counterbalance the increased intravascular pressures produced by G in all tissues below the heart. From theoretical considerations the following factors were thought to be of prime importance for maximal protection.

(1) The external pressure should be applied to as great an area of the body surface as possible, from toes to heart.

(2) This pressure should be applied in a gradient, with the pressure highest over the instep, progressively less over the legs and thighs, and still less over the abdomen, to match the graded effect of acceleration on intravascular pressure in these regions.

(3) The pressure should be applied in increasing grade as G increases.

(4) If not too uncomfortable to pilots, the pressure applied to the skin might have to be considerably greater than that dictated by the calculated or observed effects of acceleration on intravascular pressure, because of inefficient transmission of pressure from the garment to the skin, and from the skin to the deeper-lying vessels of the legs or abdomen.

The equally important practical considerations of comfort, ruggedness, and ease of upkeep modified the application of these principles, however. For instance, discomfort prevented covering the whole body by inflatable bags and compromise was required. In the second place, a gradient of pressure was found to be unnecessary in practice because a single uniform pressure on the abdomen, legs, and thighs proved to be quite as effective provided it was sufficiently high. The reason for this is still not clear. Apparently the volume of blood pooled in the legs is small compared to the amount that may be pooled at lower intravascular pressures in the large vessels of the abdomen and possibly the hepatic sinuses.

Table I compares the protection afforded by various garments and their modifications, along with the average pressure in the abdominal bladder. Effective protection ranged between 0.8 and 2.8 G, principally between 1.1 and 1.5 G. The problem was approached with two purposes in mind: first, to develop suits providing more and more effective protection; and second, to recognize the immediate tactical importance of slight protection (for example, 1 to 1.5 G) and to fabricate the simplest, lightest, most comfortable, most rugged, and most easily installed suit that would provide this modest but worth-while protection, as a compromise solution for immediate use in combat.

The figures shown in Table I were obtained from repeated tests in the centrifuge using 15-second periods of exposure and the methods of objec-

TABLE I

Average Protection Afforded by Various Types of Suits
(*Results of studies made in centrifuge of the Mayo Aeromedical Unit and that of the University of Southern California*)

Type of Suit	Number of Projects	Average Pressure in Abdominal Bladder	Average Protection in G Units	
			Mayo	U.S.C.
Gradient pressures:		lbs./sq. in.	G	G
Hydraulic suit (Franks, Canada)	12	1.63	0.9	—
Pneumatic gradient-pressure suit (U.S. Navy)	21	5.63	1.5	1.3
Single pressure:				
Narrow-bladder suit, single pressure	13	6.25	1.5	—
	12	5.00	1.9	—
Simplified type, trouser or overall	12	5.00	1.4	1.6
	18	3.50	1.1	1.1
Skeleton or cutaway type	12	5.00	1.1	—
	9	3.50	0.8	—
Arterial occlusion:				
Arterial occlusion, 4 limbs, and abdominal bladder	12	9.00	2.8	—

tive recording illustrated in Figure 30. There still remains the question whether the results obtained in the centrifuge apply to actual flying conditions in combat planes. The validity of testing in centrifuges was demonstrated by Lambert, who compared the protection afforded in the centrifuge by one of these suits with the protection given by the same suit in a combat-type plane. For this purpose the portable oscillographic unit shown in Figure 26 was employed.

A typical series of records obtained in these observations in the plane is shown in Figure 34. In a control flight with the suit on but not inflated, the relaxed, unprotected subject blacked out at 4 G. The blood content of the ear became very low and ear pulse disappeared. In a second run (B) the suit

was automatically inflated to a pressure of 1 pound per square inch per G, starting at 1.5 G. Thus, at 4 G the pressure in the suit was 2.5 pounds per square inch. At this point the protected subject had no blackout, the blood content of the ear was reduced little if at all, and ear pulse persisted throughout. At 5 G with an inflation pressure of 3.5 pounds per square inch blackout occurred once more. From comparisons of records of this type with a
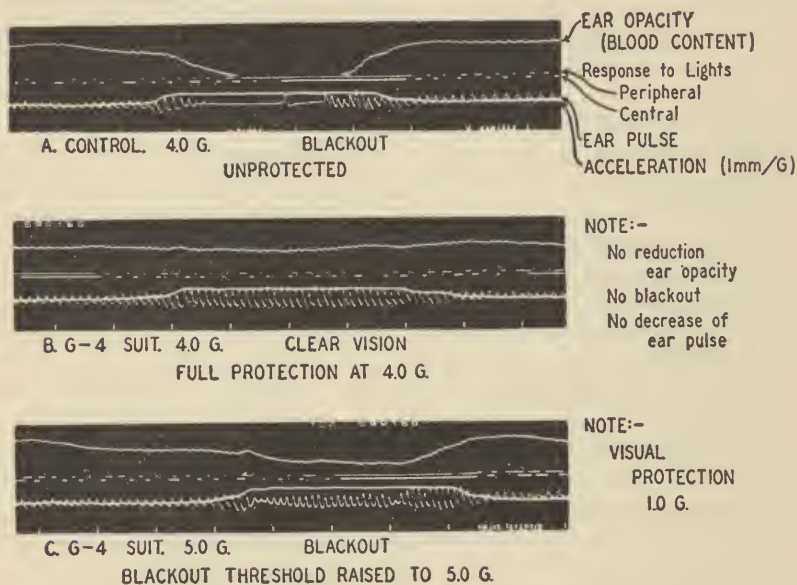
FIGURE 34. *Records taken with the portable accelerometer unit in an airplane.*

similar series of records from tests in the centrifuge in a number of subjects, it was concluded that this particular suit afforded visual protection of 1.1 G during acceleration in the airplane and 1 G in the centrifuge. This was true despite the fact that the average tolerance of unprotected subjects in the plane was 0.7 G higher than in the centrifuge. Average protection measured by blood content of the ear was 1.2 G in the airplane and 1.1 G in the centrifuge. Measured by the amplitude of ear pulse it was at least 1.6 G in the airplane and 1.5 G in the centrifuge. Measured by heart rate it was 1.4 G in both plane and centrifuge. The close correspondence between results from subjective and objective criteria and the agreement between independent tests by two groups (Table I) make these figures highly convincing.

The hydraulic suit of Franks consisted of an indistensible outer garment containing a continuous series of bags of water between the outer garment

and the skin of the legs, thighs, buttocks, and abdomen. Acceleration automatically increased external pressure on the skin *pari passu* with intravascular pressure in each location, except for the minor difference between the specific gravities of water and of blood. The protection afforded in standard tests in the Mayo centrifuge was only 0.9 G in relaxed subjects exposed to accelerational forces for 15 seconds. Somewhat greater protection was observed in the Toronto centrifuge, where test periods of 5 seconds were used. This suit had the disadvantage of requiring filling with water before each use, of restricting motion somewhat, and of being both heavy and warm in the tropics. On the other hand, it had the advantage of being a self-contained and complete unit and required no special installation in the airplane.

In considering the comparative effects of the hydraulic and pneumatic suits, it is interesting to note that immersion in water up to the xiphoid or slightly above does not wholly neutralize the circulatory effects of acceleration. In the Mayo centrifuge men in a water bath did not receive unlimited protection but only that for 0.9 G, exactly the same as that provided by the water suit. In dogs 5 G reduced blood pressure in the carotid artery to 2 per cent of normal. A water suit still permitted a fall of blood pressure to 56 per cent of normal. Immersion in a water bath was slightly more effective, but the blood pressure fell to 62 per cent of normal.

The lesser effectiveness of the hydrostatic suit may be the result of two factors: poor transmission of pressure from the abdominal skin to the splanchnic blood vessels, and the fact that the pressures that can be applied to the abdomen by hydrostatic pressure are much lower than the pressures that can, with reasonable comfort, be applied by pneumatic pressure. Studies at Randolph Field showed that an abdominal bladder inflated to 1 pound per square inch per G (50 mm. of mercury per G) increased intra-abdominal pressure only 20 to 30 mm. per G. Another observer noted that bearing down or grunting, which give more protection than some suits, could raise abdominal pressure by 60 and 100 mm. Total intra-abdominal pressures up to 300 mm. were observed in the centrifuge at the University of Southern California when the M–1 maneuver was used during acceleration.

Evidence from the Mayo unit indicates that the most important factor in protection by suits is the pressure applied to the abdomen. Pressure on the legs alone affords very slight protection but doubles the effectiveness of the inflated abdominal bladder. Thus, in one comparison inflating the abdominal bladder alone produced protection amounting to 0.7 G, the leg bladders alone 0.2 G, and both together 1.4 G. Applying pressure to the arms adds another slight increment of protection, but is generally impractical because of interference with manual control of the plane.

The figures in Table I show also the relation between the level of pressure applied to the abdomen and the grade of protection. The pressure applied

to the legs appears to be much less critical. In agreement with these findings is the conclusion that a gradient-pressure suit, applying higher pressures to the thighs and legs, offers no more protection than a single pressure applied to all three locations.

The single-pressure suit had the great practical advantage of requiring for inflation of its bladders only one valve activated by G, rather than the bank of three or more valves required by the various gradient-pressure suits. The practical problem of installing the G valve in the various types of planes will not be described here, although it required quite as much work as did the fabrication of the suit. It was necessary to arrange for a dependable and adequate source of compressed air at all reasonable altitudes and for connections between the G valve and suit that could be engaged or disengaged with a single movement of the hand.

Another advantage of the single-pressure suit was its extremely simple system of tubing and bladders. Units of this type could be built into trouser-type suits, flying overalls, or even a cutaway model. Adjustments could be made by lacings when the suit was first issued to ensure accurate fit. Liberal use of zipper fastenings permits easy donning of the garment, which can be put on rapidly over flying clothing or used alone in warm climates where a warm suit cannot be tolerated by pilots under altitudes of 10,000 feet. The protection afforded by this abridged suit (see Table I) is less than that of other models, as might be expected from the reduced coverage of the extremities, but in some situations convenience and comfort of the pilot outweigh the disadvantage of slightly reduced protection.

In ordinary use, again to ensure comfort, the bags are left uninflated until the specified G is reached, usually 1.5 G. At this point the G-operated valve, between the suit and the source of compressed air in the plane, opens and automatically increases the pressure in all the bags at the rate of 1 pound per square inch per G. Higher pressures could be used and greater protection obtained, but 5 pounds per square inch apparently represents the level at which discomfort often becomes a limiting factor.

Considerably greater protection at higher pressures has, in fact, been obtained with the so-called "arterial-occlusion" suit of the Mayo unit (Table I). In this suit the abdominal bag is employed as usual but four narrower cuffs are placed proximally on the extremities. At graded pressures up to 9 pounds per square inch, according to the imposed G, the arteries and veins of all four extremities are completely occluded. The protection afforded is higher, probably because blood returning to the right side of the heart under the influence of abdominal compression is expelled by the left side into a significantly reduced arterial bed, and a greater cephalic blood flow results.

In future studies of protective suits the effect of external pressures, low or high, on the total cross section of the arteriolar bed needs further in-

vestigation. Inflating an abdominal binder to raise intra-abdominal pressure by 30 to 40 mm. of mercury is said to reduce blood flow through the kidney by as much as 30 per cent. This must be due to increased peripheral resistance, but it is not yet clear whether this change in resistance to flow is produced simply by passive congestion or by partial compression af arterioles, with reduction in their total area of cross section. In any case, a 30 per cent reduction of renal blood flow would compensate for a decrease of 400 cc. per minute in venous return.

Two other ingenious suits may be mentioned briefly. The pneumatic lever suit devised by Herrington and Lamport contains a longitudinal, inflatable rubber cylinder, or half-cylinder, external to the suit proper and lateral to the legs. During inflation the pressure is transmitted from this lateral unit by interlacing cords to indistensible fabric encircling the legs. The graded diameter of the cylinder provides more uniform distribution of pressure to the skin of the legs, with apparently less discomfort at high pressures, than is produced by the usual pneumatic suit. The net suit devised by Wilkins consists of a corded garment with interspaces about ½ inch square. Tension from above, which can be produced by the pilot's increased weight during G, pulls the net on the bias, increases its length, diminishes its circumference, and applies an even, general pressure to the skin of the extremities.

Summary

In summary, the arterial hypotension and cerebral ischemia produced by positive radial acceleration can be reduced, but not eliminated, by self-protection, by posture, and by applying external pressure to the abdomen and extremities. The effects of these measures are often additive, so that when a suit protects the relaxed subject by 1 to 1.5 G this figure indicates only the minimum protection that can be depended on by the relaxed pilot. This minimum itself, when transformed into the advantage of retained vision at greater speed or shorter radius of turn, confers a highly significant tactical advantage. To it can be added the increments, within certain limits, of self-protection and posture for still more advantage.

The mechanisms by which these measures exert their protection appear to include, in order of importance, increase of intra-abdominal pressure, which increases venous return from the splanchnic pool; reduction of the arterial or arteriolar bed, which diverts such blood as may be expelled by the left side of the heart from the arteriolar beds of the abdomen and extremities to the cephalic arteries; and improved venous return from the extremities. More fundamental study is required to explain adequately the practical results that have been obtained by the pragmatic approach required in war research. It must be emphasized that the retention of tactical advan-

tage in the air will require constantly more efficient suits for conventionally seated pilots as the speed and maneuverability of planes increase, or else modification of planes so that they can be controlled by a prone or supine pilot.

EFFECTS OF NEGATIVE RADIAL ACCELERATION

Negative radial acceleration — that is, from feet to head in the conventionally seated pilot — is not often encountered in significant grades except in certain acrobatic maneuvers; for example, outside spins, outside loops, and inverted flight. The symptoms were well known before 1940, and little or nothing has been added since. Even tilting in the head-down position on a tilt table at -1 G produces bradycardia, embarrassment of respiration by the weight of the viscera on the diaphragm, and a feeling of facial congestion. At -2 or -3 G all these symptoms are much magnified. In addition, the eyes feel as though pushed from their sockets and red vision, or "redout," appears. Consciousness is usually retained, but mental confusion and motor inco-ordination may appear and may persist for several hours, along with severe throbbing headache. The effects on the circulatory system are the converse of those seen in positive radial acceleration. Arterial blood pressure in the carotid arteries of dogs has reached 300 mm. of mercury or more. In man the conjunctiva and facial skin may present numerous petechial hemorrhages after severe exposure.

This particularly disagreeable type of acceleration is avoided by pilots and apparently presented no immediate military problem. The greater urgency of the problems posed by positive acceleration took precedence, and practically no work was done on negative acceleration during the war years. There is at present no known means of ameliorating the effects of negative G. However, as mentioned above, the effects of radial acceleration of an airplane, whether positive or negative, can be lessened by placing the pilot in the prone or supine position with the long axis of his body at right angles to the radius of the turn. This, in effect, transforms radial acceleration of the plane to transverse acceleration of the pilot, in which direction very high G forces may be tolerated without serious symptoms.

EFFECTS OF TRANSVERSE ACCELERATION

Transverse accelerations are those directed across the body from back to front or from front to back. The far greater tolerance to transverse acceleration, positive or negative, as compared with radial acceleration, had been studied before 1940 in this country and in Germany. With pilots in the conventional sitting position, transverse acceleration up to 6 or 7 G had no subjective effect except for the sensation of greater pressure on the parts

of the body that supported the increased weight. At 8 G respiration became increasingly difficult as the thorax and abdomen were compressed. Accelerations up to 12 G produced no disturbances of vision, and except for brief inability to breathe did not seem to cause undue discomfort. In the back-to-front direction 12 to 16 G produced increased secretion of tears, with resulting blurred vision. Respiration in this position was slightly more embarrassed than in acceleration from front to back, presumably because of the greater weight of the spinal column. In animals arterial blood pressure in the carotid artery rose very slightly in a stepwise fashion and slight tachycardia was usual.

In the Mayo centrifuge 5 subjects were exposed to transverse acceleration from front to back up to 10 G for 3 to 10 seconds. Difficulty in breathing appeared at 8 to 10 G but was not distracting in short runs of 3 seconds. Pain in the epigastric region occurred between 4 and 7 G in some subjects but could be diminished by appropriately modifying the support of the shoulders, head, and hips. No dimming of vision occurred except in 2 subjects whose shoulders had to be raised for comfort to a point where the eyes were 31 cm. above the floor of the cockpit. At 10 G it was possible to roll the head from side to side and to operate a switch with the fingers.

Objective findings included slight bradycardia, increase in blood content of the ear, and, in 2 subjects, premature systoles. Ear pulse did not change significantly. It was concluded that transverse acceleration up to 10 G can be tolerated provided the head and body are adequately and comfortably supported. The head should preferably be level with the base of the heart or very slightly forward.

In the prone position transverse accelerations from back to front up to 9 G were tolerated without visual symptoms, but pressure on the chin required a special chin rest or chin cap to distribute the weight of the head uniformly over the mandible and if possible the maxilla. Dyspnea appeared at 9 G, and records of respiration showed decreased respiratory amplitude in all runs above 7 G. Comparisons on the same subjects showed that assuming the prone position would offer protection of more than 6.5 G against radial acceleration of the plane.

These observations were carried out on resting subjects, who were not required to undertake any movements other than pressing a switch. When purposive and complicated movements were attempted during acceleration, striking effects were found. In abandoning a plane falling to earth out of control, crawling across the cockpit or donning a parachute may have to be accomplished during radial G. Moreover, if planes are developed to accommodate prone or supine pilots, a certain amount of movement will be required to control the plane.

The time required for crawling a distance of 7.5 feet on a flat surface at right angles to radial G was increased 2.25 times by 1 G, 5 times by 2 G, and

10 times by 3 G; it became impossible for 3 of 5 subjects at 4 G. Similar results were observed when subjects attempted to round a barrier. It appears that at 4 G it becomes impossible to perform movements requiring transfer of body weight. It was impossible to crawl, walk, climb a ladder, or rise from a bomber seat against a force of 2 to 3 G. Donning a parachute required 17 seconds at 1 g (gravity), 21 seconds at 1 G radial, 41 seconds at 2 G, and over a minute at 3 G, and was practically impossible at 4 G.

The important implications of these studies with reference to the successful escape of fliers from a disabled plane are clear. For the future development of aircraft these observations are still more significant. Even as the prone and supine positions reduced the effects of radial G on the circulatory system, they revealed important physiological limitations of the skeletal muscles on which the pilot must depend for controlling his plane in whatever position he assumes. These limitations must by all means be taken into account from the very beginning on the designing board as new planes are planned, if engineers expect human beings to pilot the ever faster and ever more maneuverable planes of the future.

CHAPTER XXII

VISUAL PROBLEMS *

WALTER R. MILES AND DETLEV W. BRONK

W HEN THE United States entered World War I in 1917, a multitude of problems required immediate solution. For many of these there was an insufficient scientific background, and scientists had to be satisfied with empirical answers. It was clear that aviation would play a leading part in the war and that the problems of pilot selection, equipment, and maintenance were of prime importance. Aviation was the "eye of the Army," and for obvious reasons the pilot himself must have good eyes and superior visual skills. Assurance of excellent vision demanded research along many lines. Provision was made for this field of investigation by the establishment of the Department of Ophthalmology at Hazelhurst Field, Long Island, and the Air Service Medical Research Laboratories at Issoudun in France. The content and routine of the ophthalmological examination for aviators were carefully worked out, and studies were made on depth perception, color discrimination, visual fatigue, dark adaptation, and visual protective devices. This investigative program ended abruptly shortly before the Armistice in November, 1918.

In the interval between World War I and World War II, the research in vision in connection with aviation made relatively little progress, certainly, until the 1930's, owing largely to the return of specialists to civilian practice and the lack of financial support. The progress in aviation ophthalmology actually accomplished during World War I seemed more than adequate to the needs of commercial aviation, and finally the availability of pilot subjects for visual studies was greatly restricted after the cessation of hostilities.

However, in military aviation following World War I the ophthalmologist had an assured place. At all Army airfields and naval air bases, where pilot examinations were made before or during flight training or operation, ophthalmologists were in fact looked on as key men in the medical staff. At the schools of aviation medicine at Randolph Field and Pensacola, aviation ophthalmology was taught to the flight surgeons. The subjects covered included such matters as visual acuity, depth perception, ocular movements, accommodation, inspection of the eye, color vision, field of vision for form and color, refraction, and ophthalmoscopic examination. On the other hand, there was little or no emphasis on research in vision and visual problems.

* See note at end of chapter.

The major impetus making for a research program on vision from aircraft in the twenties and thirties came from the Matériel Center at Wright Field, the Naval Research Laboratory in Washington, and, perhaps even more importantly, from commercial aviation. The medical men and other scientists working with commercial companies were dealing with the selection and maintenance of pilots engaged in operations, including flights at fairly high altitudes over the Atlantic and Pacific. The effect of anoxia on vision received a considerable amount of scientific attention. In this period nutritional science developed rapidly, especially along the lines of vitamin study, and it was discovered that vitamin A influenced the process of dark adaptation. This development stimulated visual work in several laboratories and also promoted an interest in developing apparatus for measuring dark adaptation. The practical nutritional interests, therefore, supplemented those already existing in laboratories where fundamental visual research was in progress.

Eye protection for pilots was a subject of considerable importance in military aviation circles, even in connection with peacetime requirements. At Wright Field a large amount of research was done on this subject, and aviation goggles of excellent optical quality were designed and produced and generally adopted for military use. The need for wearing oxygen masks along with the goggles was not critical at this time, so that the goggles were fitted to the nose and other portions of the face without this contingency in mind. It was assumed that the goggle problem had been solved, until the experts in aviators' equipment faced the more concrete problems presented by World War II.

In October 1940, the Health and Medical Committee of the National Defense Council requested the National Research Council to constitute within its Division of Medical Sciences a Committee on Aviation Medicine, which should survey the status of the medical and physiological problems pertaining to aviation. The study of Army airfields and naval air bases that ensued revealed only a minor emphasis on the visual problem. There was some interest in the fogging and frosting of goggles and the means for combating it, but in general visual research for developing new accessory equipment or for training in visual skills seemed of minor importance in comparison with the obvious physiological needs. The Committee's recommendations following the survey contained two references to visual problems. Fogging of goggles was stated to be of prime importance in connection with mask design, and night blindness was emphasized as a serious problem calling for the development of a simple apparatus for its estimation.

The first occasion when visual problems were brought before the Committee on Aviation Medicine for considerable discussion was its meeting early in December 1940. A representative of the Navy's Department of Aeronautics presented nine problems, four of which concerned the eyes of aviators. These were distortion of vision through the windshield; fogging of goggles

and glasses; the general effect of glare from the exhaust, instrument panel, and reflections; and the effect of discharges of lightning on pilots' vision and general reactions. The Committee took no action on any of these problems, partly because they were not couched in terms of proposed contracts for research, and partly because its attention was focused on physiological problems to the exclusion of other questions. In fact, in the list of projects that the Committee had on hand at this time the subject of visual research was not even mentioned.

At the same meeting there was presented a report on night vision and the use of the adaptometer, which stressed the importance of night vision for efficient lookout work, discussed the range of thresholds disclosed by investigations in civilian laboratories, described the equipment for measuring night vision then available, and pointed out the need for simplified testing instruments to be used in the classification of military personnel. Also, a letter was read from Dr. E. J. Ludvigh of the Howe Laboratory of Ophthalmology, Harvard Medical School, which discussed the possible military usefulness of a measure of visual acuity of the human eye for objects in rapid motion and described research work that had been carried on at that laboratory.

By the end of 1940 the problems of night vision were beginning to take shape. Military leaders knew full well that in the event of war there might be a great deal of night flying. A small group of physiologists and psychologists who had worked on dark adaptation from several angles in their civilian laboratories began to press vigorously for its inclusion, in some form, as a part of the qualification for pilots, as well as for truck drivers and tank operators. Late in December the National Research Council received from General Danford, Chief of Field Artillery, an offer of the services of his artillery troops at Fort Bragg for such investigations as might prove to be necessary.

When the National Defense Research Committee was organized, it assumed responsibility for instrumentalities of warfare, including all forms of visual aids, and created groups to deal with these problems. Later the Committee on Medical Research was charged with fostering research in military medicine, which included ophthalmology and visual problems. Thus, under the Office of Emergency Management there were two channels for visual research under governmental auspices, articulated with the regular military channels but administratively outside them.

In the National Research Council a Subcommittee on Clinical Investigation had been active prior to the establishment of the Committee on Aviation Medicine. Among other problems considered by it were some in the field of vision. A small unofficial advisory group on night vision was created, and between December 1940 and February 1941, two contracts for research in and development of adaptometer equipment were granted by the National Defense Research Committee. The objectives of these contracts included mod-

ification of the Hecht-Shlaer adaptometer for military use. A portable instrument of very high quality was developed and with slight modifications was adopted by the Royal Canadian Navy and later by military organizations in the United States. Researches on several visual problems were begun in the Laboratory of Biophysics at Columbia University and in the Johnson Foundation for Medical Physics, University of Pennsylvania, and several surveys of night vision in military personnel were conducted in naval and military establishments.

Historically it is worthy of record that the Flying Personnel Research Committee of Great Britain, created in January 1939, had organized a Night Vision Subcommittee in February 1941, composed chiefly of civilian scientists. The actual organization of the Subcommittee on Visual Problems of the Committee on Aviation Medicine did not occur in the United States until May 1942, but the personnel of this subcommittee had been pursuing visual research in its military aspects for the previous two years, and had been in constant contact with the visual problems that filtered into or out of the Committee on Aviation Medicine.

Members of the advisory group mentioned above were able to initiate field researches on night vision in the period from April to June 1941. Dr. Harry R. DeSilva of Yale University, who had for some years been experimenting with automobile driver aptitude tests, was afforded an opportunity to set up such apparatus for truck drivers at Fort Bragg. A night-vision threshold measurement device was introduced into the test battery, a large darkroom with an effective light trap was arranged, a Wald adaptometer was installed, and an assistant was trained to administer the testing.

The objectives of this field study were to introduce the officers in personnel divisions to the general subject of night-vision testing; to discover if possible the incidence of marked cases of night blindness; to ascertain any difference that might exist between white and Negro troops; to correlate dark-adaptation measurements against age and intelligence, the latter represented by the number of years of schooling; to compare the dark-adaptation thresholds with certain driver aptitude tests; and finally to determine the effect, if any, of ingesting a sugar solution twenty-five minutes prior to taking the test. The results indicated that sugar did not increase night-vision efficiency.

A second field test of night vision was conducted on the U.S.S. *Enterprise*. A darkroom was prepared aboard ship and equipped with the large Hecht-Shlaer research-type adaptometer. The report of this test read in part:

No gross night blindness has been found. The best subjects examined were able to perceive light of 1/16 intensity of that barely perceptible to the poorest of the subjects. Since such a wide normal range exists, dark adaptation might well be considered in the selection of personnel for duty as night pilots, lookouts, or observers. The decrease in dark adaptability with increase in age, previously known to occur, was found in this group. Striking differences in night visual

efficiency were found between officers and enlisted men. . . . Observations on night lighting and dark adaptation are reported which point to the urgency of modifying many of the practices now in use for the illumination of ships.

The thresholds obtained corresponded well with the data taken on this same instrument in civilian laboratories in experiments on civilians.

In this same period, tests were also made at Randolph Field with the Hecht-Shlaer adaptometer on a group of men between the ages of twenty-one and twenty-six, each with sixty hours of flying time. These subjects also showed a threshold that corresponded well with normal civilian laboratory data.

The results of these three field testings were reviewed at a meeting of the Committee on Aviation Medicine in June 1941. The Air Surgeon, Colonel D. N. W. Grant, recently returned from a tour of observation in England, stressed the fact that the tests reported represented thresholds for light, and expressed the opinion that the military services were also interested in thresholds for form or pattern, or perhaps even more so. He raised the question of whether the light threshold and the form threshold were highly correlated. At this meeting there was much discussion of the probable effect of vitamin A feeding and the duration of the period of feeding necessary to influence the night-vision threshold.

The observations made by Colonel Grant in England were of material assistance to the American workers on night vision, since they gave a clear picture of actual military requirements in this field. Another military development of marked historical significance also occurred in this period. An observer from the United States Submarine Base at New London, Connecticut, made a tour of the British Submarine Service and found its personnel using an adaptometer developed by the British Admiralty Laboratories. Reports of this use and blueprints for the adaptometer were brought back by the observer to the submarine base. Rear Admiral Edwards, then in command of the base, issued an order in June 1941, requiring the testing of the men and officers of his command for dark adaptation or night blindness. A replica of the British adaptometer had been made at the base prior to this date but had not been standardized. Immediate steps were taken to organize a testing program for night vision at the base. Darkrooms with light traps were constructed and a Wald adaptometer was obtained. By the end of June, 365 men had been tested and classified.

The order for testing the night vision of the entire submarine base command at New London was, so far as is known, the first order in reference to night vision in the military services of the United States. It exercised a far-reaching influence in bringing the subject to the foreground of military attention. The report of the tests, rendered to Admiral Edwards, was sent to the Bureau of Medicine and Surgery in Washington and was seen by others. The report of the study conducted on the U.S.S. *Enterprise* had even

wider circulation. Examinations at the New London submarine base continued with the objective of measuring and classifying the full personnel. As soon as a Hecht-Shlaer adaptometer became available it was installed and comparative measurements were made with it.

The Wald and the British adaptometer each had its good points, but no one instrument then available seemed fully appropriate to the general military task. At a laboratory in Yale University development was begun of a portable adaptometer somewhat similar to the one built in 1918 at the Medical Research Laboratory at Hazelhurst Field. The new adaptometer was to exhibit a T-shaped visual target presented in different orientations. At the same time work was begun at Yale on the development of self-luminous plaques to serve as night-vision acuity targets in testing and classifying personnel. Work on apparatus for measuring night vision was also proceeding at Wright Field during the summer months of 1941. Lighting engineers at the Eastman Kodak Laboratories co-operated in building an elaborate night-vision testing unit, involving the use of successive lines of letter targets, seen as dark objects against a lighter background.

A conference on the general subject of measuring and classifying personnel in reference to night vision was held at Wright Field on August 18, 1941, and the following recommendations were adopted: that the period in the dark before measurement should be twenty-five minutes; that the size of the target should be 2 degrees of visual angle; that both flash and form targets should be used and thresholds taken; that light of daylight quality should be employed; and that the threshold should be expressed in log units, of micro-lamberts, and that further comparisons should be made of scores for flash thresholds and form thresholds on actual lookout performance under field conditions. It was also recommended that the distribution curve of the threshold scores for a population be divided into segments, designated as "excellent," "average," "poor," or "rejected" according to their deviation from the average or mean.

The results of the Wright Field conference were presented later in the month at a meeting of the Committee on Aviation Medicine in Washington, where they received further discussion and elaboration. As a result of comments made by military representatives, including Colonel Grant and Captain Poppen, it was possible for the first time to draw up a summary of statements that met with the approval of these military representatives. This summary read as follows:

(1) Night blindness is not a pressing problem, since few subjects have fallen in the category of clinical night blindness.

(2) We are not prepared at the present time to eliminate applicants on the basis of a single measurement of threshold for either flash or form.

(3) We are not prepared at the present time to categorize personnel for night duty on the basis of a single test.

(4) The improvement of illumination in cockpits as to color and intensity is of practical importance.

(5) Preliminary dark adaptation of the eyes before night missions is important.

(6) The prime need is a rough screening test which can be used on large numbers of personnel.

On this occasion preliminary work on dark-adaptation goggles was reported. It was stated that red Corning glass in such goggles permitted the wearer to perform certain visual tasks during the process of becoming dark-adapted.

By this time visual problems had been accorded first priority; they included night vision, dark adaptation, glare, and color vision. During the next three months steady progress was made with the development of adaptometers. Laboratory tests were made at Yale University demonstrating the effectiveness of the red dark-adaptation goggles, and sample goggles were distributed for service testing and further development. Work was continued on cockpit lighting and other problems, and Dr. Charles W. Bray of Princeton University was appointed by the National Defense Research Committee as a technical aide to co-ordinate the results of visual research in progress under civilian auspices. At the Institute of Optics of the University of Rochester, work was done on problems in connection with binoculars for use in airplanes. Dr. Selig Hecht finally succeeded in producing a simplified portable adaptometer, and preliminary work on testing subjects was accomplished with it.

With the declaration of war in 1941, there came immediate and strong pressure for prompt decision on scientific instruments and methods for operational application. Instrument makers were deluged with government contracts, and pressure was brought on scientific consultant groups to produce practical instrumentalities and to give advice on visual requirements. Bray proposed that a conference on adaptometers be held immediately, stressing the need of securing an instrument or instruments that would give accurate prediction of visual ability in night duty, would give reliable scores on repeated tests, would be adaptable to group testing, and could be procured promptly in the necessary number of test units. Accordingly a conference on night-vision tests was held in Dayton, Ohio, on January 25, 1942, with Bronk as chairman. There were twenty-seven persons in attendance, including representatives from the Army and Navy and the Royal Air Force and a distinguished civilian scientist from Great Britain, Dr. E. D. Adrian. The R.A.F. rotating hexagon and four other adaptometers were described and demonstrated. The improvements and developments presented included a self-luminous plaque that could be rotated to place the target figure in different positions and filters to provide four brightness levels; a lantern type of unit in which the lighting on a test window 6 inches in diameter was

graduated by means of an iris diaphragm; and an improvement of the latter by Dr. Paul Klopsteg in which the light intensity was controlled by circular wedges and the test lamp standardized by means of a photocell.

The conference recommended that the Hecht adaptometer be sent to Randolph Field to be tried on candidates, together with the luminous-plaque adaptometer, improved as much as possible, for comparison of results of the two units. It was also recommended that fifty of the instruments proposed by Klopsteg be ordered at once so as to be available for use at the various reception centers and in the Navy for actual rough classification of cadets and other personnel. The conference asked various experts to collaborate in improving the instruments so far developed.

The outcomes from these several developments are matters for record in the military history of the war. Suffice it to say that the Eastman AAF Night Vision Tester was completed, gave satisfaction in group testing, and was employed at several large Army Air Forces centers. A self-luminous plaque adaptometer with one filter was adopted for general use for the Navy, and several hundred of these were manufactured and used on shipboard and elsewhere. At the Aeromedical Laboratory at Wright Field a simple self-luminous plaque unit, to be held in the hand of the examiner at a fixed distance from the subject, was developed and widely used by flight surgeons in the armed forces. A similar unit was developed at the School of Aviation Medicine at Randolph Field. The Hecht adaptometers, both the research model and the portable model, were used chiefly as research equipment, with the exception that the portable model, with slight modifications, was adopted for classification use by the Royal Canadian Navy.

The Committee on Aviation Medicine completed its first year of service in October 1941. The Committee by this time was really functioning as a committee on visual problems, as well as for other groups of problems. Bronk and Walter R. Miles had served as sponsors in this field. With both men on the same committee the optical field received strong emphasis, and there were gradually fostered the beginnings of a Subcommittee on Visual Problems, which held its first meeting on May 19, 1942. The personnel of this subcommittee consisted of Dr. Selig Hecht, Columbia University; Dr. Walter R. Miles, Yale University; Dr. Robert Newell, Stanford University; Dr. Brian O'Brien, University of Rochester; Dr. H. K. Hartline, University of Pennsylvania (Secretary); and Dr. Detlev W. Bronk, University of Pennsylvania (Chairman). Throughout the war this committee played an important role by recommending contracts on visual problems and supervising research.

The deliberations at the first meeting were concerned with six topics: the brightness of the night sky, the use of glaring lights as a means of offensive and defensive warfare, color-vision tests, rod and cone vision at night, effects of choline on night vision, and night-vision tests. O'Brien and Hartline were instructed to prepare a summary of studies of night sky brightness and its

relation to visual physiology; Hecht and Hartline were to prepare a program of investigation on the use of glaring light in collaboration with O'Brien and the National Defense Research Committee section working on this problem; and Hecht was asked to investigate tests of color vision and to devise a new test that could be recommended to the services. The committee was in agreement that choline, in the light of recent experiments, could not be considered of value for improving the night vision of normal observers.

The subsequent work of this committee carried out under the Committee on Medical Research was in three general areas: selection standards, training, and the development of protective measures and visual aids for military operations.

Methods for measuring the night visual capacity of military personnel have already been discussed. Adaptometers of the several types, notably the Hecht-Shlaer instrument and the Eastman AAF instrument, were extensively employed in central training establishments, and the radium-plaque instruments were used for field purposes. Serious defects of night vision were, however, seldom found. Nevertheless, Army Air Forces personnel and certain other categories of military trainees were tested for exceptional or relatively poor qualifications in this regard, so that the proper persons might be selected for exacting night duties as the need arose. Actually, the practical use of this information for combat assignments in the field proved difficult, except in extensive cases. The significance of this effort lay more in the emphasis on the facts of night vision, which were translated into training and operational and protective procedures and formed the groundwork for later practice in selection. It is probable that for future military operations at night men will be selected on the basis of exceptional night vision. Certainly those who are deficient should be rejected, just as those who have poor visual acuity are now rejected for day operations in combat.

It is significant that the outstanding work of Hecht, Wald, and others in night vision during the prewar years only slowly gained wide recognition in military circles. The obvious lesson is that civilians and military personnel must work together more closely during peacetime if the knowledge of the scientist is to be put to use quickly in wartime.

Tests for color vision have long been an important part of the selection qualifications for military aviators. The most satisfactory and significant tests have, however, been widely questioned. Accordingly, Hecht, at the request of both the Army Air Forces and the Navy, carried out extensive tests of selection procedures. Improved lantern tests were thus developed and — of more immediate use in the services — the significance and practical value of the Ishihara Test were better defined.

The requirement of normal color vision for aviation personnel presented a difficult selection problem, for the elimination of 5 per cent or more of potential fliers on the ground of defective color vision was undesirable in the

days of manpower shortage. Accordingly, Hecht was requested to co-operate with the Army Air Forces Training and Proving Ground Commands in determining the color-vision requirements for pilots, navigators, bombardiers, aerial gunners, and radio operators. On the basis of these findings it was possible to redefine somewhat the color-vision standards for air crew acceptance, with a resultant saving of personnel who would otherwise have been rejected.

It has been said that as the importance of night vision in aerial warfare became recognized there was a growing appreciation of the value of training in methods of seeing at night. This was early emphasized by Hecht and Miles and was readily accepted by the medical services. It was more difficult to persuade those responsible for training and operations. This was due in part to a general unawareness of the differences between day and night vision, and in part to the fact that aerial operations at night had not often been carried out in the past and were not an extensive part of the early American campaigns in World War II. On the other hand, the requirements of the Royal Air Force and the Royal Canadian Air Force were severer in this regard, so that they resorted to extensive training programs. Also, in the United States Navy, notably under Captain C. W. Schilling of the Submarine Service, excellent training activities in night vision were developed in the early months of the war.

Finally, the pattern of our aerial warfare changed so that there was greater need for night operations. Accordingly, in 1944 the Army Air Forces requested the Committee on Medical Research to develop a program for training in night vision. In his capacity as Co-ordinator of Research for the Air Surgeon and Chief of the Division of Aviation Medicine of the Committee on Medical Research, Bronk served in a liaison capacity between the military agencies and the civilian scientists in this effort. Hecht, Miles, and Hartline surveyed the existing training practices in the Navy and the British and Canadian air forces, and subsequently made recommendations regarding the factual content of a course of instruction and the design of a demonstration trainer.

Following these suggestions, experts from the Johnson Research Foundation of the University of Pennsylvania developed such a training device, which derived many of its features from a similar instrument designed by an officer of the Royal Canadian Air Force. By means of this instrument moving silhouettes of planes were caused to move across a screen illuminated so as to simulate a night sky, and many of the facts of night vision were revealed to groups of trainee-observers. The relative visibility of stationary and moving objects, the value of scanning procedures, the effects of glaring lights, the importance of dark adaptation, and the value of red light for the protection of night vision were thus presented, and practice in the use of proper procedures was made possible. After appropriate field trials of this

training device at the Columbia, South Carolina, Army Air Base, the instrument was redesigned. Thereupon eleven units were constructed in the Johnson Research Foundation and fifty more were constructed by the Army Air Forces.

In order that the training in the principles and practice of night vision should be most effective, the Army Air Forces charged their commissioned aviation physiologists with the conduct of this program. So that they in turn might be adequately qualified for their duties, the Committee on Medical Research conducted three one-week courses in visual physiology for sixty of these officers at the Johnson Research Foundation. These courses were notable examples of scientific co-operation. To meet an urgent military need the Committee and the Army Air Forces combined in the effort, leaders in the field of visual physiology from various universities gave instruction, and the material of instruction was drawn from classical peacetime research and from recent wartime investigations.

The schedule for these courses was as follows:

Outline of Course in Visual Physiology
for Aviation Physiologists

MONDAY	Hour
Introduction — Dr. Detlev Bronk	1030
Status and Regulations Concerning Night Visual Training in the Army Air Forces	
The Physiology of Vision — Dr. Selig Hecht	1130
Histology of the Retina	
Photochemistry of the Visual Mechanism	
Dark Adaptation	
Spectral Sensitivity of the Retina, etc.	
Lunch	1200
The Physiology of Vision — Dr. Selig Hecht	1300
TUESDAY	
Physiology of the Pupil — Dr. Irving Wagman	0900
The Effects of Oxygen Lack on Vision — Dr. Selig Hecht	1030
Lunch	1200
Demonstration of Visual Tests for Anoxia in Chamber "Flights"	1300
WEDNESDAY	
Suggested Lecture-Demonstration — Lt. Charles C. Wilson	0900
The Night-Vision Projection Trainer — Dr. Lorus J. Milne	1100
Lunch	1200
Administrative Problems Relating to the Night-Vision Training Program — Lt. Charles C. Wilson	1300
Aircraft Recognition Program in the A.A.F. — Major Davidson	1400
THURSDAY	
Psychological Problems Concerning Vision — Dr. Walter Miles	0900
Advice on Training Methods	

Outline of Course in Visual Physiology
for Aviation Physiologists
(*Continued*)

Following this instruction the aviation physiologists returned to their several posts in the Training Command, the Troop Carrier Command, and the Air Transport Command, where they presented in a few hours the simpler facts of night vision to flying personnel and gave them practice in how to see at night. Others were assigned to combat units in theaters of operations in order to improve the efficiency and safety of personnel flying at night. Unfortunately, this training program was developed late in the war and had little effect on actual operations; indeed, night flying played a minor role in the operations of our air forces up to the end of the war. Nevertheless, we should have been prepared for the visual requirements of such operations had the war continued. Furthermore, many of the basic facts of night vision, discovered or formulated by Committee on Medical Research scientists, had been taught to Army and Navy air force trainees in the altitude training programs that began in 1942.

Among the facts emphasized in the altitude training programs as being important in high-altitude operations was the influence of anoxia on night vision. Before the war, investigations had shown that lack of oxygen reduces the ability to see dimly illuminated objects. Accordingly, this became a matter of importance to the air forces. Committee on Medical Research scientists formulated the results of previous work, and research was carried on to

determine the degree of visual loss at various altitudes. On the basis of this work, the air forces of both the Army and Navy issued directives requiring the use of oxygen for even low-altitude operations at night. This was desirable, for even at 5000 feet the impairment of night vision was measurable. Hecht also developed so-called Contrast Discrimination Charts, which demonstrated to flying personnel, in the course of their altitude-chamber training, the loss of night vision under various degrees of anoxia.

As our military operations reached out to desert regions and tropical coral beaches, another hazard to night vision was revealed. It was, of course, well known that exposure to a bright light for even a few seconds would reduce the ability to see dimly illuminated objects for some minutes thereafter, until the process of dark adaptation had again restored the normal sensitivity of the retinal rods. Indeed, both British and American investigators had experimented with glaring lights as a means of offensive and defensive night warfare. They soon found, however, that there were practical difficulties in directing into the eyes of a flier a light of sufficient intensity to leave him seriously incapacitated for any appreciable time. On the other hand, fliers who spent their days exposed to bright sunlight were found to suffer a long-persistent deterioration of night vision and a slowed capacity for dark adaptation.

In a series of careful experiments in the field and in the laboratory, Hecht showed results that he reported as follows:

A single exposure of two or three hours delays the onset of rod dark adaptation by ten minutes or more, and slows the process itself so that the normal night-vision threshold is not reached for several hours afterward. After repeated daily exposures to bright sunlight, the delay in reaching the normal threshold persists overnight, and the threshold after complete dark adaptation rises higher each day for about ten days and remains at a higher level after that. This elevated threshold corresponded to about 50 per cent in visual acuity, range of visibility, contrast discrimination, and in the frequency of picking up a target when it was barely visible. This chronic effect did not disappear even after ten days of protection from sunlight.

Because of these discoveries, flying personnel who were much exposed to glaring sunlight in tropical and subtropical regions and in the snow of the North were warned to protect their eyes with appropriate sun glasses. Thus they were made more efficient for their night combat duties.

The specifications for sun glasses for such protection were among the problems relating to the physiological characteristics of optical instruments, which occupied much of the attention of visual experts. In this particular instance there was the question of the transmission characteristics required to give maximal protection against the harmful effects of sunlight, while permitting adequate visibility. From known data, from experiments, and from confer-

ences between physiologists, designers, and military procurement officials, an appropriate compromise was achieved.

Another optical problem to which physiologists made valuable contributions was the improvement of binoculars and recommendations for their more effective use. These instruments have a vital function in night operations, for by increasing the size of the retinal image and collecting more light into the eye they make possible the detection of objects that could not otherwise be observed.

In a series of experiments directed toward the improvement of the design of binoculars for this purpose, Hecht showed that under intensities of illumination corresponding to twilight the critical factors that determine the visibility of a target are the magnifying power, the exit pupil diameter of the sighting instrument, and the degree of contrast between target and background. Under twilight conditions magnification proved to be most important, and the more magnification there was available the greater was the range of visibility. With failing light, however, the exit pupil of the sighting instrument proved to become increasingly critical; the larger the pupil the more effective was the sighting instrument for increasing the range of visibility. Particularly at lower illuminations, the contrast between target and background was found to be of prime importance in limiting visibility with sighting equipment. Because of these considerations Hecht recommended that for operations at twilight, sighting instruments should be selected with the highest magnification that could be obtained with a 7-mm. exit pupil. To increase the size of the exit pupil beyond that value was not found to be useful.

Another related line of investigation was that of Wald at Harvard University on the proper focus-setting of binoculars for use in dim lights as contrasted with high illuminations. It had been reported that observers tended instinctively to set variable-focus optical devices more negatively in very dim light than in bright light. The question posed by the services was whether this was advantageous to the conduct of night warfare. By careful tests, Wald showed that this was a proper procedure. The setting of optical devices about 0.4 diopters more negatively in very dim than in bright light was advantageous because of the chromatic aberration of the eye. Indeed, for some observers a negative setting of 1 to 3 diopters may be advantageous because of involuntary accommodation.

A third research project on the design and use of binoculars for night operations was conducted by Hartline and his associates. In terms of the physiological characteristics of the eye, he was able to define the optical characteristics of the instrument so as to ensure the greatest tactical usefulness. Optimum magnification in relation to size of exit pupil and objective was thus specified. On the basis of these determinations, Hartline stated that a 10-power instrument was better suited for night use than the standard

7-power instrument, even though it became necessary to sacrifice the size of the exit pupil and the brightness of the retinal image in order to keep down the bulk of the instrument. These investigations also emphasized unsteady holding of the instrument as one of the major limitations on its usefulness for night operations.

This matter of steadiness was a major consideration in the employment of binoculars for night bombers and especially for night fighters. To meet this difficulty much work was done by OSRD and service personnel on various types of vibration-proof mounts, and Hartline devised a simple rubber frame for the binoculars, which thus rested firmly against the supraorbital ridge and face. In the end, the use of binoculars for aerial combat at night proved to be so advantageous that they were fast becoming standard equipment.

An important problem already mentioned briefly was the preservation of the dark adaptation of personnel operating aircraft (and other military equipment) in the presence of lighted panels of instruments or while reading maps and charts and oscilloscopes. Such procedures require a high visual acuity that is available only in the fovea, but the high brightness levels necessary for the functioning of the foveal cones impair the adaptation of the highly sensitive night visual mechanism of the retinal rods. It was well known that red illumination minimizes this undesirable effect, for the reason that the sensitivity of the rods is only slightly greater than that of the cones where red light is employed, whereas for any other color the sensitivity of the rods greatly exceeds that of the cones. The principle of retinal physiology underlying this effect has been well known since Parkinje's original observations, more than a century ago, on the shift in the region of maximum brightness of the spectrum toward short wave lengths as illumination is reduced.

The possibility of applying this principle to military problems was suggested to Hartline by A. V. Hill, of London, while the former was serving as scientific attaché to the British Embassy in 1940. After trials under practical conditions, Hartline submitted reports to the Navy Department recommending the use of red lighting for the illumination of instruments and enclosures in order to give maximum protection to operating personnel. In the Navy red battle lights were adopted for surface and subsurface craft; also considerable effort was expended in this arm of the service in designing systems of red illumination for the instruments of night fighter aircraft.

In a further extension of this type of investigation our workers collaborated with the Bureau of Aeronautics of the Navy in tests of various types of panel-illuminating systems, by using a Link trainer under actual operating conditions. These tests were carried out in the Planetarium of the Franklin Institute, where the influence of the operating lights on the delay in spotting a faint target against a night sky could be measured precisely.

The effect on night vision of viewing radar oscilloscopes was another phase

of this general problem and was investigated by similar methods. "Red trace" oscilloscope screens were tested, but it was found that such special devices were unnecessary, since adequate protection of dark adaptation, combined with ample signal visibility, could readily be obtained by the use of a red filter over the conventional orange P7 screen. This project was part of more extensive consultative services furnished by the Committee on Medical Research to the Radiation Laboratory at the Massachusetts Institute of Technology on visual problems involved in the design and use of radar equipment. An important outgrowth of this work was the development by Hartline of a daylight viewing hood for radar oscilloscopes in Navy fighter planes, which cut off the sunlight from the tube-face while permitting the pilot to look out through the sides of the hood at his other instruments.

Among the instrumental aids to vision that found wide acceptance was the dark-adaptation goggle developed by Miles in collaboration with Captain Leon Carson, of the Navy. By the wearing of these goggles, made of red plastic with suitable spectral transmission, before the performance of critical night duties, dark adaptation was preserved and personnel went into action with good night vision.

These same principles of visual physiology found application in relation to the muzzle flash of guns. Committee on Medical Research workers served as advisers to the Ordnance Department on such problems. Data were furnished on the visibility of flashes of light under various conditions of ambient illumination. Also, assistance was given to Professor Ladenburg, of the Aberdeen Proving Grounds, in developing a photoelectric photometer that evaluated the light output of muzzle flashes in accordance with the distribution of spectral sensitivity of the retinal rods. Field tests were also conducted to determine the recovery of dark adaptation following the exposure of a gunner's eyes to bursts of fire from 50-mm. cannon with various types of propellents and flash-hiders.

There were also important visual problems involved in the design of rifle sights, especially for use in low illuminations. Warden made valuable contributions along these lines by carrying out for the Navy an experimental analysis of the visual task involved in small-arms marksmanship. After studying the sighting scores obtained in aiming Garand and Springfield rifles with various types of front and rear sights, in bright and in low illuminations, he found that the aperture type of rear sight (Garand) was far superior to the open or notch type (Springfield) when the size was properly adjusted to the level of illumination. The standard Garand sight, with an aperture of 2.035-mm. diameter, was found to be extremely accurate at a level of illumination approximating sunset on a clear day. It was, however, greatly inferior to a sight with an aperture 4.5 mm. in diameter at a level of illumination corresponding to thirty-five to forty minutes after sunset on a clear day, which is the lowest level of illumination in which sighting with a

rifle is possible. According to Warden's conclusions, the best combination of sights for dim illumination is: for the rear sight, a closed type with an aperture of 3.5 mm.; for the front sight, a post or bead type with a face about 2.9 mm. wide, coated with a bright substance.

SUMMARY

From the foregoing account it will be apparent that military operations, especially in the air, posed many visual problems. In the solution of those problems, in the development of selection and training programs, and in the effective design of optical instruments that would satisfy the physiological requirements of the human eye, the Committee on Medical Research had the services of an able group of scientists in the field of vision. They translated their fundamental knowledge into useful practice, quickly and with remarkable ingenuity.

NOTE: References to the work of certain individuals contained in the authors' manuscript have been eliminated and certain changes and omissions have been made in order to conserve space.

CHAPTER XXIII

MOTION SICKNESS

PHILIP BARD

W ORK on the problem of motion sickness was initiated by a subcommittee of the Committee on Aviation Medicine of the Division of Medical Sciences, National Research Council. The Subcommittee on Motion Sickness was established in July 1942, in response to a letter to Dr. Lewis H. Weed from Brigadier General Hillman. That officer pointed out that the problem of motion sickness was one of increasing importance to the Army in connection with the use of small boats, airplanes, and gliders in landing operations, and requested that the Division of Medical Sciences undertake a study of this disorder with a view to discovering some means of decreasing the loss of efficiency resulting from it.

At its first meeting the Subcommittee was confronted with abundant indications of the urgency of the problem with which it was to deal. The testimony of representatives of the Ground Forces, the Army Air Forces, and the Navy and reports from England and Canada suggested that the incidence of seasickness in amphibious training and combat operations had been high, resulting at times in serious loss of efficiency; that airsickness had become a problem of some magnitude in the selection, training, and proper elimination of air force personnel, particularly navigators, bombardiers, and gunners; and that operations then anticipated, in which very large numbers of troops would be carried by air and by small surface craft, might be attended by a serious degree of incapacitation from motion sickness. In retrospect it can be said that this picture of the problem was not an exaggerated one.

STATUS OF THE PROBLEM IN 1942

DEVELOPMENTS BEFORE THE WAR

The literature on motion sickness that appeared before World War II contains amazingly little factual information bearing on either the genesis or the therapy of this common disorder. Most of the publications on the subject were devoted to uncritical considerations of a number of possible etiologic factors or to reports of uncontrolled or inadequately controlled tests of measures for the prevention or cure of motion sickness.

The outstanding development had been the obtaining of evidence that the vestibular apparatus is an essential factor in the production of motion sickness. The pioneer observation of William James, reported in 1882 and later confirmed by others, that deaf-mutes with nonfunctioning labyrinths are immune to seasickness, and the classical animal experiments of Sjöberg (1931), which demonstrated that after bilateral labyrinthectomy previously susceptible dogs fail to exhibit any signs of sickness when exposed to motion,[1] had gone far to establish the view that motion exerts its effect through one or more of the sense organs of the nonauditory labyrinth. The well-known fact that disease or artificial stimulation of the labyrinth may result in nausea and vomiting also supported the belief that motion sickness is of vestibular origin. The calculations and observations of Wojatschek (1909), Quix (1922), and Sjöberg (1931) had focused attention on the probability that, while angular accelerations may produce the signs and symptoms of seasickness, the sickness evoked by the motions of ships and boats is the result of exposure to linear accelerations, chiefly in the vertical plane. Thus the tentative conclusion had been reached that the commonest form of motion sickness depends on utricular stimulation.

The prewar literature, and the assumptions of many who began to work in this field at the beginning of the war, tended to place a good deal of emphasis on the importance of extralabyrinthine factors in the genesis of motion sickness. There were suggestions that movements of heavy viscera, changes in the distribution of blood in the body, alterations in the activities of the gastrointestinal tract, olfactory stimuli, visual influences, and general somatic stimuli are either indispensable or strong contributing factors. The hypothesis that psychological or psychopathologic influences are of primary importance received much attention. Many of these suggestions, theories, and claims seemed reasonable enough and therefore demanded exploration, for not one had been supported by adequate experimental evidence. There had to be kept in mind the possibility that should any one of them prove correct, the establishment of that fact might be of the utmost significance in the rational development of a control of motion sickness in military operations.

In respect to drug therapy, both prophylactic and curative, it can be said that no claim of the effectiveness of any pharmacologic agent or combination of agents made before the war was convincing. Most of these claims were based on the impressions of physicians who had, from time to time, treated small numbers of seasick patients, most of whom doubtless represented the most susceptible group in the general population. Scarcely any tests were controlled by the giving of placebos, and one looks in vain for data that could meet the most rudimentary statistical requirements. After a perusal of this

[1] This fundamental observation was confirmed in work carried out during the war under the auspices of the Subcommittee on Seasickness of the Canadian National Research Council.

literature it was easy to appreciate the cogency of the statement of one writer that "given a patient who is subject to real sickness, and weather conditions that cause a ship to roll, pitch, yaw, vibrate, and shimmy, I am convinced that the only real and complete cure is to sit under a big tree."

The drugs that had been used most widely belonged to two groups, the belladonna alkaloids (atropine, hyoscine, and hyoscyamine) and the group of hypnotics and sedatives (particularly various barbiturates, bromides, and chlorobutanol). The latter substances had obviously been chosen because of their depressant action on the central nervous system. The use of the belladonna alkaloids apparently had its origin in the vague clinical impression that many sufferers from seasickness are vagotonic and therefore may be benefited by a peripheral blocking of parasympathetic discharges to visceral effectors. The fact was generally overlooked that all these substances have central nervous effects.

In view of the prewar lack of any really rational basis for the use of hyoscine in the control of motion sickness, it is interesting to note that work done during the war by Australian, Canadian, English, and American investigators has shown quite conclusively that this drug, given in appropriate doses, is an effective preventive of motion sickness. It is also true that the preventive tentatively adopted by the Ground Forces of the United States Army and the only one, so far as this writer knows, actually used by them in combat operations contains amytal, hyoscine, and atropine. It was developed, before the outbreak of the war, by a medical officer of the Army, Lieutenant Colonel L. L. Barrow, who had used it in an attempt to reduce the high incidence of seasickness encountered among Army personnel traveling by ship over the often stormy waters separating the Hawaiian Islands. Subsequent extensive tests of this preparation, known as Motion Sickness Preventive, Army Development Type (M.S.P., A.D.T.), demonstrated its effectiveness.

Developments During 1942

In the early months of 1942 work on motion sickness as a problem in military medicine was under way in England. A good deal of attention was devoted to exploring the use of swings in motion sickness studies, a procedure that had apparently originated in Russia some fifteen years before. The English work was directed toward the problem of selection of air-borne troops, adaptation to motion, and the control of sickness during landing operations from air and sea. The efficacy of a number of proprietary remedies was tested, and as a result it was concluded that hyoscine was a safe and effective preventive. It had also been decided that in the control of seasickness during amphibious operations medication offered far more than any possible form of "preselection."

Also early in 1942, there began in Canada the most extensive program of inquiry into the cause, incidence, and control of motion sickness that the war years produced. This was carried out by naval, military, air force, and civilian investigators under the auspices of the National Research Council of Canada. Several machines designed to duplicate the motions of ships were built and used in the study of both human and animal subjects. Later, when it became evident that the simple swing was just as effective and would permit many different workers to apply the same form of motion, this device was adopted. It was established that in a swing the subject is affected almost solely by radial accelerations. By the close of 1942 the Canadian groups had reported results indicating which of a large number of drugs tested should be further studied as agents likely to be of use as preventives, had physiologically and biochemically surveyed the reactions of men and dogs to motion, had confirmed the important fact that in dogs bilateral labyrinthectomy puts an end to susceptibility, had explored the matter of adaptation, and had looked into the problem of correlation of airsickness with swing sickness and histories of motion sickness. These early studies were of great service in shaping the course of later investigations, not only by the Canadians themselves but also by workers in the United States.

Meanwhile in Australia some very interesting developments were taking place. As early as January 1942, the marked effect of head position on the development of motion sickness had been noted. Attention was also directed toward the matter of preselection by swing tests and the question of emotional factors in airsickness. A later finding of no little significance was that suppressive doses of antimalarial drugs have no appreciable effect on susceptibility to motion sickness.

In the United States one of the earlier attempts to study and control motion sickness as a military problem was initiated at Camp Edwards, Massachusetts. At the United States Naval Hospital in Chelsea, gastrointestinal, neurologic, and psychiatric studies of chronic seasickness were begun. Later the subject was taken up at the Naval Air Station in Pensacola; this led to one of the more conclusive demonstrations of the effectiveness of hyoscine as a preventive of airsickness. Interest in the problem developed in the Airborne Command (a branch of the Ground Forces), and a few observations and tests of drugs were made during operational flights. Early in the summer of 1942 research was instituted at the Army Air Forces School of Aviation Medicine, Randolph Field, Texas. Before the close of 1942 this group had contributed much information bearing on the validity of a number of procedures (swing tests, labyrinthine function tests, gastrointestinal findings, and determination of onset of cold sweating) as means of detecting susceptibility. The continuation of this work through the war years contributed much to our understanding of the problem of motion sickness.

ACTIVITIES OF THE SUBCOMMITTEE

The Subcommittee's recommendations were based on the total picture of the problem revealed by its various activities. Members of the Subcommittee were guests at conferences on motion sickness held by the Canadian workers. Its own meetings were attended by delegates from Canada, England, and Australia and by representatives of several service groups. Members visited military and naval stations where work on the problem was in progress and took part in the organization and conduction of field trials of preventives. The Subcommittee distributed progress reports on work being done by a variety of agencies. Thus, while it served as a clearinghouse for information, it gained knowledge and perspective.

Early in December 1942, a broad plan for future studies was outlined. This included basic studies of the etiology of motion sickness in man and animals, selection of personnel by determinations of susceptibility, and therapeutic studies involving drugs and other measures. As time went on it became more and more apparent that the most pressing need was a drug or combination of drugs that, given by mouth, would prevent motion sickness in a majority of susceptible persons for periods up to eight hours and would not produce undesirable side-effects. In February 1943, the Subcommittee reported that the most promising preparations for the prevention of motion sickness were hyoscine, Vasano (a commercial preparation containing hyoscine and hyoscyamine), phenobarbital, and M.S.P., A.D.T. A year later it was agreed that, although work on the thiobarbiturates (V–9 and V–12) and on a combination of prostigmine and atropine should not be discontinued, efforts to obtain comparative tests should concentrate on M.S.P., A.D.T., hyoscine alone, and the Royal Canadian Navy Seasickness Remedy (composed of hyoscyamine, hyoscine, and niacin). Shortly, a slightly encouraging trial of these three took place when representatives of the Royal Canadian Navy, the Bureau of Medicine and Surgery of the United States Navy, and the Subcommittee obtained records on 4048 subjects carried by about thirty LST's during a total of fifteen different days of travel in convoy. A careful statistical analysis of the data allowed the conclusion that the three preventives were effective in reducing sickness rates under moderate conditions of roughness. Obviously more satisfactory tests were needed, and they were soon obtained. By September 1944, new developments had led to the placing of emphasis on finding an optimal combination of V–12 and hyoscine and comparing its effectiveness with those of other preventives.

Throughout its active life the Subcommittee was assailed by the difficulties that attended the securing of adequate field tests of preventives. Many of the tests carried out involved groups of subjects too small to permit conclusive results, even under the most favorable conditions, or suffered from other

methodologic defects. At an early juncture it became clear that decisive results, applicable to the solution of the most pressing military aspects of the problem, could be expected only when the number of subjects used and the sickness rates in control groups were high enough to yield statistical reliability. Amphibious training operations in which large numbers of troops were exposed to motion simultaneously and under similar conditions (the same type of boat and so forth) obviously presented the most desirable conditions.

The most important duty of the Subcommittee was to initiate and encourage experimental work and clinical tests. Apart from recommending that certain work be done under OSRD(CMR) contracts, it fostered and aided studies in several quarters, especially a number of field tests of preventives carried out by officers of the Army and Navy. The Chairman, Dr. Derek Denny-Brown, initiated trials of a number of pharmacologic preparations on small vessels of the Coast Guard often exposed to the roughest weather. He arranged for tests of several preventives to be carried out on transports, and was instrumental in having tablets of hyoscine placed in kits aboard life rafts as a means of combating seasickness.

PRESENT STATUS OF THE PROBLEM

The incidence of sickness depends on the character and duration of the motion and on the susceptibility of the persons exposed. Human beings (and certain species of animals, particularly the dog) display all degrees of susceptibility, and probably no normal person is wholly immune. About 10 per cent of normal persons may be classified as highly susceptible. Adaptation, a result of repeated exposures to motion, does occur, but it is minimal or absent in the very susceptible. It is conspicuous in the training of many aviation students.

From the military point of view, the most serious aspect of the problem is encountered in the transport of large numbers of men to or through combat areas in small ships and boats or in aircraft. Here selection of personnel is impracticable and control must depend on preventive measures. In the training of specialized flying personnel, where much time and effort may be wasted because of airsickness, a means of early detection of chronically susceptible persons is still urgently needed. The use of single swing tests or questionnaires has proved to be a relatively inefficient means of selection. Probably a method that determines capacity to adapt will effectively detect most of the chronically susceptible persons. Such vestibular function tests as the usual Barany chair test and caloric tests fail to predict susceptibility.

The usual symptoms of motion sickness are, approximately in the order of their appearance, drowsiness, pallor, cold sweating, nausea (usually ushered in by some degree of epigastric awareness), and vomiting, which

may or may not occur. The degree of incapacitation produced varies greatly from person to person and may bear little relation to the occurrence of emesis. Headache and dizziness are vague symptoms that do not occur with any regularity and cannot be used as specific criteria. True vertigo and nystagmus are not evoked by the motion of planes, boats, ships, swings, or elevators but occur when sickness is produced by angular acceleration. Changes in blood pressure and heart rate are insignificant during the development of sickness. Some increase in pulmonary ventilation may occur; it is chiefly encountered in highly susceptible persons. No significant changes in blood composition have been found except as the result of other symptoms, such as alkalosis after hyperventilation and alkalosis and ketosis after long-continued vomiting. Gastric hypotonia and hypomotility are often produced by motion; they occur more frequently among the susceptible than among the relatively immune, but there is no acceptable evidence that they exert any influence on the development of nausea, vomiting, or other symptoms of motion sickness.

The development of motion sickness depends on exposure to movements possessing special characteristics. No motion of constant velocity produces sickness; a long series of changes in velocity (accelerations) is required. With increases in acceleration level (g value) there is an increase in incidence up to a certain moderate level, beyond which further increases are accompanied by a reduction in sickness rate. The effectiveness of a motion also depends on the time interval between accelerations, the duration of the component accelerations, the total cycle duration, and the total energy per cycle.

It is beyond dispute that labyrinthine stimulation is essential for the production of motion sickness. The effective motion may involve purely linear or purely angular accelerations or a combination of these. The consensus of opinion is that in the motions of ships and swings linear accelerations are the effective stimuli, and there are several good reasons for the belief that the sickness produced results chiefly from utricular stimulation. When sickness is evoked by angular accelerations, the symptoms include vertigo and nystagmus (not seen in seasickness, airsickness, or swing sickness), and this fact indicates that the cristae ampullares of the semicircular canals are the sense organs activated (utricular stimulation does not produce nystagmus). The possibility remains, however, that in the genesis of both seasickness and airsickness angular as well as linear accelerations are involved, although there can be little doubt that the latter are the more important.

The central nervous mechanisms activated in motion sickness as a result of labyrinthine stimulation include of course the various segmental centers of the brain stem (chiefly bulbar) directly responsible for the bodily changes characteristic of motion sickness. That these segmental centers are subordinate to the specific central mechanisms involved is shown by the fact that susceptible dogs are rendered wholly immune by decerebellation or by re-

moval of only the vestibular portions of the cerebellar cortex. The role played by the cerebral cortex is important but remains to be elucidated.

The question of the importance of extralabyrinthine factors in the etiology of motion sickness has received much attention. The available evidence shows that visceral displacements, gastrointestinal changes, and circulatory alterations exert no significant influence in the genesis of motion sickness. Although, in the absence of body motion, optical disturbances may produce nausea, the question of the precise influence of visual stimuli in the production of sickness due to motion remains uncertain. Visual influences may either increase or decrease susceptibility, aggravate or ameliorate the disorder. The view that susceptibility is related to emotional instability and neurotic traits is based on general impressions and on the psychiatric examination of a few small groups of highly susceptible persons. One of several weaknesses of this interpretation is that nothing is known regarding the incidence of neurotic traits in the portion of the population that is least susceptible. It is contradicted by failure of one careful study to reveal any evidence of a correlation between personality disorders or general psychosomatic complaints and susceptibility to seasickness. The readiness with which a highly susceptible person develops conditioned responses to odors, sights, and so forth is well known; it can scarcely be regarded as evidence of a neurotic constitution or predisposition. There is convincing evidence that the giving of a placebo in the guise of an effective preventive does not influence the incidence or the severity of seasickness or airsickness.

The first indisputable evidence that prophylactic drug medication is effective was obtained in World War II. This came as the result of carefully controlled experiments carried out during amphibious training operations, in a few longer sea trials, in aviation training programs, and in swing tests. The agreement is general that hyoscine, in a dose of 0.6–0.8 mg., protects from 50 to 60 per cent of susceptibles over a period of at least eight hours without producing side-effects that are undesirable from a military point of view. It has not been established whether combinations of hyoscine with atropine or hyoscyamine are either more or less effective than hyoscine alone. There is some indication that the addition of a barbiturate may enhance the protective action of hyoscine. A compound designated as V–12 (ethyl-beta-methylallyl-barbituric acid) has emerged as a preventive of some potency, but its effectiveness in comparison to that of hyoscine remains in doubt. It now seems fairly certain that benzedrine, prostigmine compounds, thiamine, nicotinic acid, and pyridoxine do not give protection. The important problem of cure was necessarily neglected during the war. There is very little evidence that any effective preventive or any other drug will control motion sickness once it has developed.

Some degree of control by nonpharmacologic means seems feasible. The induction of adaptation by exposures to subnauseating degrees of motion is

a possible measure, but the apparent specificity of the process of adaptation limits its application. A fact established during the war is that the incidence of swing sickness is enormously affected by the position of the head, regardless of that of the body, and that this effect is explicable in terms of the spatial relationship of the utricular receptors to the uniformly fluctuating force changes. The practical application of this disclosure suffers some limitation by the fact that the effective forces of the motions of various craft are apt to be exerted in more than one plane.

INCIDENCE OF MOTION SICKNESS [1]

In Amphibious Training Operations

In his studies of the effects of preventives, Tyler obtained data on the incidence of sickness evoked by exposures of one to three hours in small landing craft. In the thirty-six groups, totaling 3133 unselected young men, who received placebos (lactose in capsules) the total sickness rate varied from 11 per cent, when there were gentle swells, to 60 per cent, when the seas were moderately rough. In the former instance only one fifth of those affected were severely sick (severe nausea, with or without vomiting); in the latter, one half. Since in the course of this work it was shown that the giving of a placebo does not affect the incidence or severity of sickness, these rates may be taken as indicating the general level of susceptibility to the kinds and degrees of motion experienced. In four experiments on 303 untreated controls the rates varied from 30 to 43 per cent. These figures agree with British incidences ranging from 15 to 70 per cent when troops were carried in small boats. They lend credence to unofficial reports that in certain combat landing operations the incidence approached 100 per cent.

In Experimental Studies

Wendt, using his "wave machine" (see below), found that in tests of twenty minutes' duration the incidence of vomiting ranged from 7 to 53 per cent, depending on the characteristics of the motion used. Spiegel produced sickness within eight minutes in 75 per cent of the unselected subjects exposed to the action of his rotating-tilting device (described below).

Effect of Food and of Time of Day

The general impression that ingestion of food influences, in one way or another, susceptibility to motion sickness received no support when Tyler failed to find that, short of dietary indiscretions that would evoke illness in a still person, either the quantity or the kind of food eaten affects the incidence of seasickness. Wendt's two series of experiments involving 450

[1] This and the following sections deal only with work done under contract.

subjects revealed no reliable evidence of a relationship of incidence of sickness on the vertical accelerator to mealtime or to the period of the day. These observations support the earlier conclusion of Manning and Stewart based on their study of swing sickness.

METHODS FOR EXPERIMENTAL PRODUCTION OF MOTION SICKNESS

Work under contract led to the development of two new devices for the experimental production of motion sickness. The wave machine of Wendt is a hydraulically driven elevator with a sealed, soundproof, temperature-controlled cab, which moves up and down in a shaft 18 feet in length. It is essentially a vertical accelerator in which wave-form, acceleration level, velocity, and amplitude can be varied. It was designed for the study of the characteristics of a motion that make it nauseating and the effects of various physical and physiological conditions on the development of motion sickness. It has proved capable of inducing severe sickness.

Spiegel and his collaborators designed a rotating-tilting machine or chair, which proved highly effective. When a subject is rotated in the usual way in a Barany chair, changes in velocity occur only at the start and end of the rotation, and this type of movement is not very effective in evoking symptoms of sickness. The design of the chair used by Spiegel makes use of an old observation that moving the head during rotation in any plane other than that of the rotation quickly brings on nausea and vomiting. The chair is so arranged that with each turn the head of the subject is tilted in either the sagittal or the frontal plane.

CHARACTERISTICS OF MOTIONS THAT MAKE THEM NAUSEATING

Work done at Wesleyan University by Wendt and his colleagues constitutes the most extensive attack yet made on the problem of determining the features of a motion that make it nauseating. They used the wave machine described above and thus confined their analysis to movements in the vertical plane. Their subjects were 477 naval aviation cadets. Exposure was for twenty minutes or until vomiting occurred. The subject sat blindfolded, with his head in the upright position and with the cab temperature at 86° F.

Wendt was led to examine the effects of varying the time interval between accelerations by the common observation that vehicles and movements that yield large accelerations with brief phases are rarely nauseating, whereas those having low accelerations and long phases are likely to be extremely productive of nausea and vomiting. The first test of the hypothesis thus suggested was a comparison of the effects of four waves in which the interval

between equal accelerations at top and bottom was made to vary by changing the duration of a period of constant velocity (400 feet per minute) inserted in the middle of each wave. In the different waves this period was 0.22, 0.68, 1.12, and 1.6 seconds, the cycles per minute 32, 22, 16, and 13, and the amplitudes 4, 7, 10, and 13 feet. As the temporal separation of the accelerations was increased the subject was exposed to fewer and fewer of them and less total work was done on him. The 16-cycle wave with only half the rate of power expenditure but twice the duration of the 32-cycle wave produced six times as much sickness. The other waves were intermediate in effectiveness. Evidently the wave of longest duration (13 cycles) represents a recession of the optimum condition. These results confirmed the hypothesis that the time characteristic of a motion rather than its violence is the feature relevant to motion sickness.

A second investigation of the same hypothesis dealt with the question whether the significant variable is total wave duration or duration of the period between accelerations. Three wave types were obtained by changing not only the period of constant velocity in the middle of the wave but also the velocity itself (the greater the duration the less the velocity) while holding the wave-cycle duration constant at 22 cycles per minute. Peak values of acceleration were held constant. The intervals between accelerations were varied by varying the length of time of the application of the acceleration. The total energy per cycle (measured by peak velocity) increased as the interval between accelerations decreased. It was found that an increase in time between accelerations from 0.68 to 1.12 seconds, with a reduction in total energy per cycle, resulted in a significant decrease in sickness. It was therefore concluded that when total wave duration is held constant, the total energy per wave is a more potent factor than is time interval between accelerations.

A third study was devoted to a determination of the effects of acceleration level. Four wave types were obtained by controlling the rate of change of velocity and so altering the frequency and the total wave amplitude (between extremes of 4 and 9 feet). The total energy per wave was kept at a constant value (mid-wave velocity of 400 feet per minute). The fastest wave with the highest acceleration level (32 cycles, 0.65 g) produced the least sickness (13 per cent), while a wave of moderate frequency and moderate acceleration (22 cycles, 0.36 g) gave maximum sickness (53 per cent). Two slower waves at lower acceleration levels (16 cycles, 0.25 g, and 13 cycles, 0.20 g) were somewhat less effective (rates of 43 and 40 per cent). The results showed that nausea and vomiting are most effectively evoked by waves of moderate frequency and acceleration level. As regards the effects of frequency and g value, they are in accord with those obtained in a Canadian study by Fraser and Manning of the characteristics of the motion of a swing that make it nauseating.

The purpose of a fourth study was to find out whether sickness production is a function of wave frequency or of duration or magnitude of component accelerations. Five waves, all having the same total energy, were used. One, like the waves previously described, was a symmetrical acceleration wave. Four were asymmetrical; the amplitude, duration, and g value of the acceleration differed in the top and bottom halves. The cycle duration of two of the asymmetrical waves was the same as that of the sickness-producing symmetrical wave used, while that of the other two was almost identical with the duration of the most nauseating wave of the third study. Consequently all the asymmetrical waves had intervals between accelerations that might be expected to induce high sickness rates if cycle frequency is important. The top and bottom halves of the unequal waves had different acceleration levels. In two the different g values were ones that when used in symmetrical waves were relatively non-nauseating. Each of the other two waves was composed of a relatively ineffective half-wave and a nauseating half-wave. The sickness rates obtained were very close to what would be expected if the half-wave character, rather than wave frequency, determined the result. The authors suggest that effective motion acts on some resonant mechanical system with a natural period of three or four seconds, but point out that all structures in the body likely to be involved are short-period, heavily damped systems. They therefore recommend that acceptance of the conclusion be deferred until further evidence is forthcoming.

The effectiveness of the rotating-tilting machine of Spiegel depends on the fact that it imparts to the labyrinths of the subject a motion in which the velocity is rapidly and regularly altered. At the rate of rotation used (30 revolutions per minute) there is an angular acceleration and deceleration every two seconds. The rhythmic tilting of the head in the sagittal or frontal plane during rotation at constant velocity in the horizontal plane causes the angle between the plane of rotation and the plane of the horizontal or vertical semicircular canal to increase or decrease with each turn, thereby exerting the same effect on the endolymph as a regular change in velocity of rotation. Tilting the head in the frontal plane gave a somewhat higher incidence of sickness (66.7 per cent) than tilting it in the sagittal plane (51.7 per cent). The highest sickness rate (80.8 per cent) was achieved when, during rotation, a succession of sagittal head movements was followed by a succession of head movements in the frontal plane. The incidence of sickness was not affected by tilting the body as well as the head. There can be no doubt that the sickness produced is primarily due to repetitive stimulation of the cristae ampullares by angular accelerations. Definite post-rotatory nystagmus was observed in all subjects. The tilting of the head of course gives rhythmic up-and-down movements of the labyrinths, but their amplitude is small and it is therefore doubtful that they are of significance. The over-all effect of the combined motions on the utricular maculae is not

easily pictured; doubtless they are affected by centrifugal force. There is a possibility that their stimulation played some part in the genesis of the sickness.

An observation made during this work bears on the question of the influence of vision. The 183 subjects used in the tests described were rotated with their eyes closed, but experiments were performed on 30 subjects who kept their eyes open. It was found that the incidence of sickness tended to be less when the subject fixed his gaze on a lamp that participated in all the movements of the head than when he watched three stationary lamps placed around the apparatus. It is not clear how this effect is related to Manning and Stewart's finding that the incidence and severity of swing sickness are augmented when vision is excluded by closure of the eyes or restricted by enclosing the swing.

CENTRAL NERVOUS MECHANISMS INVOLVED IN MOTION SICKNESS

An effective motion must evoke some specific pattern of discharge of nerve impulses from the receptors of the labyrinth to the brain. The maintenance of this afferent bombardment for some length of time is obviously a condition essential for the development of sickness. The production of the disorder appears to involve a most striking example of temporal summation of afferent impulses. The answer to the question of the particular physiological characteristics of the central machinery concerned is one that would have important bearings on the development of a rational therapy. The first step in the experimental approach to this aspect of the problem is to determine the location of the central mechanisms that are specifically related to the development of motion sickness. A part of this step was taken by me and my collaborators, C. N. Woolsey and R. S. Snyder. The results are outlined in the following paragraphs.

The role played by the cerebral cortex was examined by determining the effects of cortical ablations on the susceptibilities of dogs whose performances on the swing had been established by weekly tests. It was found that the following cortical removals did not alter susceptibility to the emetic action of motion: bilateral temporal lobectomy; bilateral removal of the visual cortex; removal of both frontal poles (containing the somatic motor and sensory areas); removal of all the neocortex except both frontal poles; and ablation of all the cortex on one side and removal of all the neocortex on the other except the frontal pole. The question whether the development of the objective symptoms of motion sickness depends on any part of the cerebral cortex can be answered only by determining the effects of complete decortication, a procedure that was not successfully accomplished. Babkin and Schachter found, however, that nearly complete decortication rendered a pre-

viously sensitive dog nearly wholly immune to swinging during a survival of nearly three months.

The fact that the best-known suprasegmental representation of the vestibular receptors lies in the cerebellum led to an examination of the possibility that this part of the brain is concerned in the production of motion sickness. A dog that, in eleven swingings at weekly intervals, had invariably vomited within eight to twenty-five minutes and had always shown profuse salivation was subjected to removal of all the cerebellar cortex and was studied over a period of seventeen months. In fourteen tests of sixty minutes each and one of two hours the animal failed to vomit, and the small amount of salivation observed was associated with the occurrence of panting. Equally striking results were obtained in a series of dogs from which only the nodulus, uvula, and pyramis were removed. Control experiments showed that the removal of adjacent portions of the cerebellum, including the pyramis, did not reduce in any way the sensitivity of the animals to motion. The conclusion was drawn that the nodulus and uvula contain neural mechanisms that are prepotently involved in the genesis of motion sickness in dogs. These two parts of the cerebellum, together with the flocculi, comprise the vestibular portion of the cerebellar cortex. The results of these experiments leave uncertain the role of the flocculi in the production of motion sickness.

In the course of this investigation evidence was obtained indicating that susceptibility to motion sickness is independent of susceptibility to certain other emesis-producing agents and conditions. It was found that the former is not correlated with the susceptibility of dogs to the emetic action of apomorphine. The inference of this result was borne out by the observation that the dog rendered immune to motion by decerebellation showed a normal sensitivity to the drug, and by the fact that the animals in which partial cerebellar removals had abolished the emetic effect of swinging occasionally vomited in their cages after eating.

PREDICTION OF SUSCEPTIBILITY

The sixth study at Wesleyan University consisted of examination of the relationship of the responses of 477 subjects on the vertical accelerator to their susceptibility to motion, as indicated by questionnaires administered two to ten weeks before exposing them to the machine. The histories provided information about experiences on boats and in automobiles, trains, streetcars, buses, airplanes, and various amusement devices. On the basis of their histories the subjects were divided into three groups according to apparent susceptibility. These groups were equally represented in the exposures to each of the fourteen wave types used. All other known important variables were counterbalanced or controlled. A reliable and moderately high relation between history of sickness and actual incidence of experimentally produced

sickness was found. The sickness rates of susceptibles, intermediates, and nonsusceptibles were 45, 24, and 14 per cent, respectively. It was concluded that sickness induced by the machine is due to factors common to the production of sickness by other means, and that prediction of sickness in military situations might be achieved by the use of such a questionnaire. While this conclusion is in accord with the results of other carefully conducted studies, the available evidence shows that the use of histories alone cannot be relied on as an efficient means of selecting or rejecting candidates for such activities as those of an air crew.

Spiegel and his co-workers endeavored to ascertain whether results obtained on the rotating-tilting machine might serve to indicate susceptibility to seasickness or airsickness. Histories of previous experiences of the subjects were taken before the tests were run. Nearly all of those classified as moderately susceptible and about half of those judged to be nonsusceptible were made sick by the most effective form of stimulation (see above). The weaker method (rotation combined with sagittal head movements) showed a little more capacity to select. In view of the fact that the machine produced sickness chiefly, if not entirely, through the effects of angular accelerations, while linear accelerations are the more important stimuli in airsickness and seasickness, there exists reasonable doubt of the relevancy of this study.

EFFECT OF BODY POSITION

In the course of his extensive studies on prophylactic medication, Tyler, using the control groups that received a placebo, found that the position assumed by troops in landing barges during standard ship-to-shore amphibious training operations exerted a pronounced effect on sickness rates. For purposes of security and concealment the practice was to require the men to assume a crouching posture (resting on one knee) as soon as they entered the barge and to maintain it during the entire operation. In nine experiments involving 899 men who crouched from ship to shore the average incidence of sickness was 30 per cent (range, 25–42 per cent), and 10.1 per cent were severely sick. In contrast to these figures were those obtained when 1220 men, in fifteen experimental groups, were permitted to stand except during the final ten-minute run from the line of departure to the beach; the average sickness rate was only 11.7 per cent (range, 5–19 per cent), and a mere 2.2 per cent were severely sick. The obvious effectiveness of this procedure led to its adoption in amphibious training on the Pacific Coast.

An explanation of the higher incidence associated with the maintained crouching position would probably include the operation of several factors. The most likely one is lack of a visual influence present when the men crouched in the barges. The crouching position kept their heads below the gunwales and thereby prevented any visual orientation to the abnormal mo-

tion; they were in essentially the same situation as the subjects of Manning and Stewart who, swung sitting in an enclosed swing with their eyes open, showed a much higher incidence of sickness than subjects swung in the same position with eyes and swing open. If the effective movements of the landing craft were chiefly in the vertical plane, the difference can scarcely be attributed to the influence of head position. There is evidence that body position *per se* exerts no effect on susceptibility.

PROPHYLACTIC MEDICATION

Effect of Placebos

A few reports, none of them convincing, have suggested that the giving of a placebo reduces the incidence of motion sickness. Studies of airsickness among navigation cadets by Lilienthal (United States Navy) and by P. K. Smith (Army Air Forces) revealed no evidence that this procedure affected the incidence. The most extensive and convincing examination of this question was carried out by Tyler. In four experiments involving 563 unselected young men, the incidence of seasickness during shore-to-shore amphibious training operations averaged 35 per cent (range, 30–43 per cent) in the four untreated groups and 34 per cent (range, 26–46 per cent) in the four groups given a placebo (lactose in capsules) before the test and *told that it was an effective remedy*. Further, the incidence of severe sickness was practically the same in the two sets of four groups (total rate of 13 per cent in the untreated, 15 per cent in the placebo-treated groups). These results are of considerable value in showing that suggestion is not a factor of importance in determining sickness rates in groups of persons unselected as regards susceptibility or previous experience. They add substantially to the bulk of evidence that psychic factors are of minor significance in the genesis of motion sickness.

Field Tests of Preventives

In the summer and fall of 1943, Bard and his colleagues collaborated with the Engineer Amphibian Command in several tests at Cape Cod and in Florida. The results were not satisfactory. Early in 1944 it was arranged that tests be made by Tyler during extensive amphibious training operations along the California coast. During the latter half of that year, thanks to the long Pacific swell, the large amount of material available, and the development of a method that met statistical requirements, data were obtained that constitute the most satisfactory body of available evidence on the problem of drug prophylaxis in seasickness.

More than 15,000 unselected troops were studied during ship-to-shore

operations in small landing craft. A random distribution of placebo and drugs to be tested (all in capsules) was practiced, and from one to three hours after the capsules were taken the men were distributed in the barges in such a way that each boatload of twenty to thirty men was made up of approximately equal numbers from each experimental group. After returning to the transport the men were interrogated (no leading questions were asked) and classified in four categories as follows: not sick; moderately sick (mild nausea but no vomiting); severely sick (severe nausea, with or without vomiting); and incapacitated. The following nine drugs or combinations of drugs were tested:

Hyoscine (0.6 mg.).
M.S.P., A.D.T. (amytal, 130 mg.; hyoscine, 0.4 mg.; atropine, 0.3 mg.).
Royal Canadian Navy Seasickness Remedy (niacin, 200 mg.; hyoscine, 0.3 mg.; hyoscyamine, 0.8 mg.).
"V-12" (ethyl-beta-methylallyl-thiobarbituric acid) (doses of 4, 5, and 6 gr.).
"V-12" (4 or 5 gr.) with hyoscine (0.6 mg.).
"V-12" (4 or 5 gr.) with hyoscine (0.3 mg.) and hyoscyamine (0.8 mg.).
Amytal (130 mg.).
Prostigmine Bromide (15 mg.) with atropine (1.2 mg.).
Prostigmine Bromide (15 mg.) with syntropan (150 mg.).

With the exception of the preparations containing prostigmine, all the medications tested were found to be effective. A statistical analysis by Dr. Margaret Merrell was made of the results of thirty-six experiments on some 12,000 subjects carried out between June 1 and December 5, 1944. The effectiveness of a remedy was expressed as the ratio of sickness rate for remedy to sickness rate for placebo on individual boat trips made under a wide variety of sea conditions. This ratio was found to maintain its level over a series of placebo rates ranging up to 30 per cent. This analysis showed that the first six remedies listed above gave sickness rates approximately 40 per cent of the placebo rates observed for the same trip; that there was no evidence that any one of the first three is more effective than the other two; and that the three containing V-12 were possibly somewhat less effective than the first three. On the basis of separate tests Tyler has concluded that the barbiturates, when given alone, are effective only when the corresponding placebo rates are below 30 per cent, whereas hyoscine affords a 60 per cent protection when the placebo rate is as high as 53 per cent. Amytal was tested in only one experiment. The two prostigmine-containing preparations were without demonstrable prophylactic action in trials in which the placebo rate was 21 per cent and M.S.P. reduced the incidence to 7 per cent.

This work has added much weight to the evidence that hyoscine is an extremely effective preventive of motion sickness. It has not indicated that the addition of atropine, hyoscyamine, or a barbiturate adds any further protection.

MISCELLANEOUS OBSERVATIONS

Effects of Sickness on Performance

Among the studies of Wendt and his collaborators was one in which the subjects, immediately on vomiting on the vertical accelerator, were given a performance test. The four tests used were the Mashburn Complex Coordinator, running through sand and weaving around obstacles, a 60-yard dash, and a dart-throwing test. The first of these was the only one in which the subjects displayed a statistically reliable deficit, and this was small (4 per cent). It was concluded that under the conditions of these experiments sickness to the point of vomiting has very little effect on performance. It was pointed out that since the exposure to motion was relatively brief (not more than twenty minutes), the conclusion is of limited application.

Investigations of the Labyrinth

Spiegel undertook experiments directed toward finding a practical means of depressing or paralyzing the labyrinthine receptors. He found that in cats, but not in dogs or man, paralysis of the labyrinth could be produced by electrophoresis of cocaine solutions from the external auditory canal. A similar effect was obtained in cats and dogs by injecting local anesthetics through the drum into the middle ear. In another investigation local cooling of the ear in cats and rabbits exerted a depressing effect on tonic labyrinthine reflexes but not on post-rotatory nystagmus.

Spiegel, Oppenheimer, and Wycis obtained evidence that the reflex depression of arterial pressure evoked by rotation may originate in the vestibular receptors for tonic reactions as well as those concerned in kinetic reactions.

CHAPTER XXIV

ANOXIA AND OXYGEN EQUIPMENT

G. A. MILLIKAN

IT IS probable that the problems of anoxia and of oxygen equipment reached their pinnacle of importance in the period of World War II. The reason for this is clear. There are three strata in the physiologist's atmosphere. Below about 10,000 feet, where most of the air combat of World War I was conducted, oxygen is superfluous for most operations. From a height of two miles to about eight, oxygen is first a boon and then an absolute necessity. At still higher altitudes, even pure oxygen is insufficient to maintain man in an efficient state, and more stringent measures must be taken, such as cabin pressurization or elimination of the human crew. In the years from 1939 to 1945, when the vast majority of combat flying was at altitudes of 10,000 to 30,000 feet, the task of supplying the flier with oxygen was an essential one for our over-all strategy. Had the twin problems of equipment development and of crew training not been vigorously and effectively attacked, this country's entire bombing program over Europe would have failed.

The boundaries between the three layers of the atmosphere are not sharp in terms of altitude, although they are precisely definable in terms of function. They are rather marginal fringes, whose extent under various applied conditions formed the principal subject of oxygen investigations by the Committee on Medical Research during the war. The lower of the two fringes was directly concerned with an important practical military question, "At what altitudes should oxygen be used?" The answer had ramifications not only in service regulations but in strategy, equipment design, equipment procurement, and emergency procedures in case of oxygen lack. The results were abundantly applied in combat operations. The upper of the two marginal fringes, where even pure oxygen begins to fail man, was beyond the limit of normal combat operation, but it was in the forefront of interest, for it might have erupted at almost any moment into a position of great importance. It was a principal task of our research organizations to keep well ahead of current operations. Approximately half of the projects dealing with oxygen and anoxia were aimed directly at boosting this upper boundary above the 40,000-foot limit, without recourse to the weighty or cumbersome solution of a complete pressurized enclosure. Many of them were concerned

with the combined effects of anoxia and other factors such as lowered baro-
metric pressure or acceleration, and therefore had many points of contact
with problems of decompression sickness and of blackout.

Linking together the high, oxygen-breathing region with the low, air-
breathing region was the common denominator of the human body and the
constancy of its needs. From the high- and low-level studies emerged a
fairly consistent and comprehensive picture of respiratory exchange in man.

ALTITUDES DEMANDING THE USE OF OXYGEN

In attempting to answer the apparently simple question of what altitudes
require the use of oxygen, the research organizations scored both success
and failure. The armed services early in the war set the level of 10,000 feet
as that above which oxygen should always be used. Although it was known
that departures from sea-level values for alveolar oxygen tension and arterial
oxygen saturation could be detected at altitudes below 5000 feet, it had not
been possible to demonstrate that these involved any reductions in functional
ability. In view of the weight and complexity of oxygen equipment, the
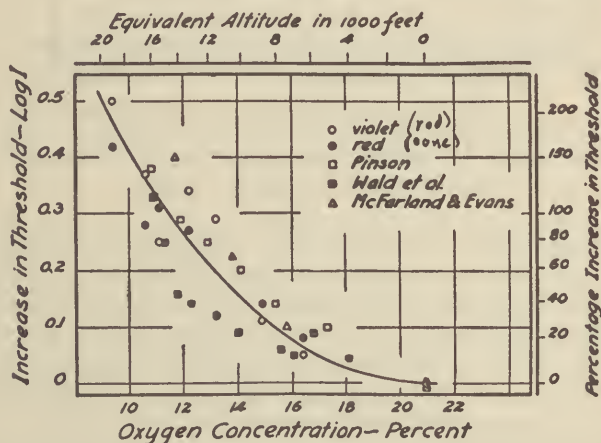
FIGURE 35. *Visual threshold as influenced by equivalent altitude.*

services wished to place the official oxygen altitude as high as was consistent
with optimal performance. Thanks to researches made both before and dur-
ing the war, it is now established that a significant rise in the visual
threshold of the dark-adapted eye and a reduction in contrast discrimination
at low illuminations take place at 5000 to 8000 feet (Fig. 35). This loss of
visual efficiency disappears at higher illuminations (Fig. 36). As a result of

this demonstration, the service ruling was modified so as to require the employment of oxygen from the ground up on all night flights. This constituted the success.

We have failed, however, either to confirm or disprove the wisdom of the 10,000-foot ruling for daylight operations. On the whole, it has been vindicated by a vast amount of actual experience, but no conclusive data have been contributed from the laboratory. The difficulty lies in the unsatisfactory nature of performance and psychological tests in evaluating the effects
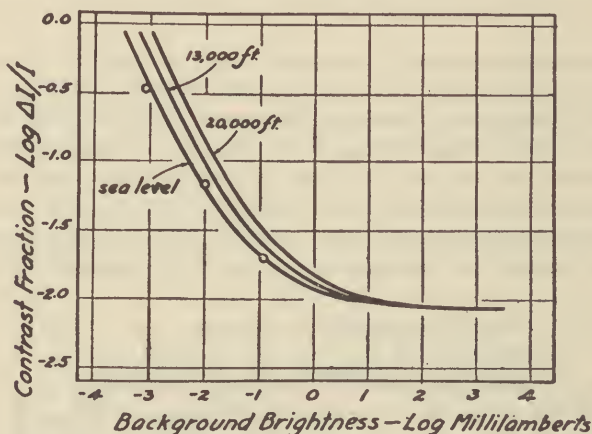
FIGURE 36. *The relation between just perceptible contrast,* ΔI/I, *and the prevailing intensity.*

of mild anoxia, because of the wide range of individual responses. Statistical studies of large numbers of subjects indicate significant degrees of impairment at about 12,000 feet, and it is agreed that the oxygen level should not be higher than this. Even at 10,000 feet, a significant shift to lower frequencies has been demonstrated in the electroencephalographic spectrum (a mean drop of about 0.2 cycles per second), and although this shift is indicative of a functional change, it has not yet been correlated with a definite degree of impairment. Exposures to this same altitude for several hours a day in experiments carried out at the University of Chicago showed a marked tendency to sleepiness, irritability, moodiness, and boredom, from which the investigators concluded that flying at 10,000 feet should not be permitted without oxygen. Unfortunately, we do not yet have even an approach to an objective and quantitative measure of fatigue or sleepiness (excretion of 17-ketosteroids showed no significant shift), although the problem of fatigue was of overwhelming importance in long bombing missions at altitude. This was a problem for which the war's urgency did not speed us to a solution.

EVOLUTION OF THE DILUTER–DEMAND OXYGEN
SYSTEM

The coming of the war found our air forces with oxygen equipment that was not well adapted to the demands soon to be placed on it. The Army "A–8" system was a great advance over the "pipestem," which it was replacing in 1938 and 1939. However, it worked on the constant-flow principle, requiring for economical operation frequent adjustment by the flier according to his altitude and state of activity. Furthermore, it had not been designed for conditions of extreme cold or for economical operation at very high altitudes, and it included a cumbersome breathing bag at the chin of the flier. The major burden of replacing this system by one that could be used effectively on massed high-altitude bombing raids by rapidly trained crews fell properly to the services themselves, yet our investigators played no inconsiderable part in the development, first in the initial stimulus that they gave to a new idea (giving the first practical demonstration of the principle to be made in this country), second by providing a large portion of the basic physiological data on which regulator and mask specifications were based, and finally by establishing the principles of mask leakage required for determining the optimum compromise between protection and economy in the safety-pressure regulators that were beginning to come into use at the close of the war.

The body's adjustments to varying activity are complex, but none is more perfectly adjusted or better adapted to its task than the regulation of respiration: the quantity of gas one breathes is the amount one needs, and this is true at altitude as well as on the ground. However, the *composition* of the air that one should breathe in order to maintain normalcy changes with altitude and not with activity. Physiologically, there are thus two independent variables that should determine the supply of oxygen to the flier: total gas flow, as determined by the natural respiratory reflexes, and oxygen fraction of the gas supplied, as determined by altitude. In two of the earliest papers circulated by the Committee of Aviation Medicine it was shown that an economical and fully automatic system could be obtained by combining a suction-operated demand valve with an air-oxygen mixing device controlled by an aneroid bellows. An apparatus operating on this principle was constructed at Cornell Medical College and demonstrated at Wright Field, where it was shown to be capable of automatically maintaining safe arterial saturations at simulated altitudes up to 35,000 feet. The oxygen expenditure was about the same as that of the older form when the latter was manually adjusted. This instrumental segregation of function corresponding to the two different physiological needs has been incorporated in all later developments (Fig. 37). There are other incidental, but important, advantages of the new

system. In its basic design it is better adapted to resist cold than the system it replaced, since inspired and expired air pass through separate channels. Furthermore, as altitudes are approached at which pure oxygen is required, the diluter-demand system becomes relatively much more economical than the constant-flow type.

After the first successful demonstration of the diluter-demand principle had been made, the different lines of development were brought under
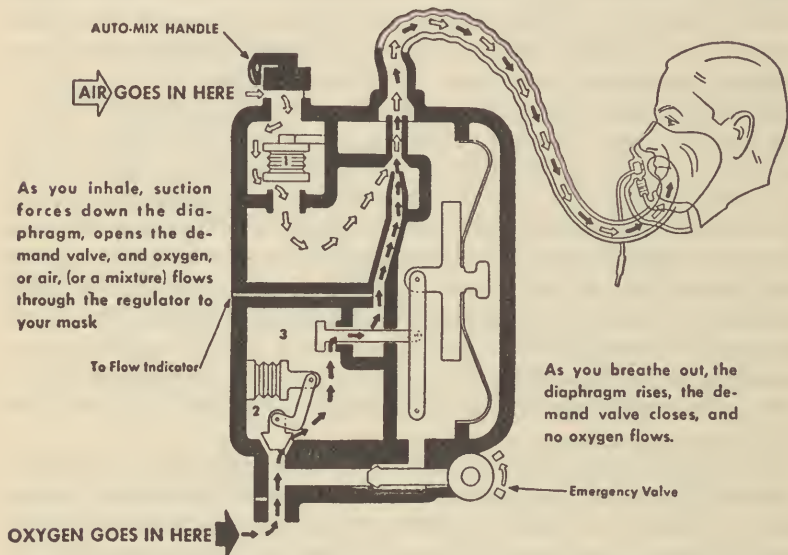
FIGURE 37. *Diagram showing the mode of operation of the diluter-demand oxygen system.*

the direction of the Army and Navy Air Forces, and the role of our investigators became progressively reduced as the production stage was approached. About this time, however, the Harvard School of Public Health developed the first American oxygen mask to be used with the new type of regulator. In its revised forms (AIO and AIOR), this mask was produced and used in large quantities, until replaced in the last years of the war by a design of Bulbullian, which, however, retained certain AIO features.

Although the role of our investigators in introducing the present oxygen apparatus was a real one, one should not minimize other convergent lines of development. Demand valves had long been used in mine-rescue equipment; an aviation model, without the economizing diluter, was being fitted to certain Navy aircraft at the outbreak of the war. The Germans, unknown to us, were already producing an automatic diluter-demand regulator, a sample of which, captured by the British, was sent to this country in the

spring of 1941. From it the American Pioneer regulator adopted the Venturi principle for its air-oxygen mixing valve. Quite independently, our Air Reduction Company was developing a completely different regulator, based, however, on the same principle of operation. Very soon, these different threads were brought together under Army and Navy direction, and there emerged the two different types of A–12 regulator, which promptly went into large-scale production, and with which the vast majority of American combat aircraft, both Army and Navy, were fitted throughout the war.

Once the principle of the newer oxygen supply system was established, the major OSRD(CMR) contribution to the development of current equipment was in the collection and presentation of physiological data on which specifications could be based. Much of this material was collected in the Handbook of Respiratory Data in Aviation, published early in 1944. Revisions in specifications were recommended in numerous conferences.

All oxygen systems are beset by the dangers of mask leaks. If these are compensated too generously, the oxygen cylinders will quickly be drained, and a subtle balance must be struck. A comprehensive theory of mask leakage was developed toward the end of the war and applied to the diluter-demand oxygen system. This showed that the optimum compromise between safety and economy could be obtained if the regulator was adjusted to operate under very slight positive pressure (so-called "safety pressure"). The most recent regulators, which were being tested for service use at the close of the war, were designed to operate in this way.

A number of accessory oxygen devices were submitted to the services from time to time by OSRD(CMR) contractors. Most of them arose as by-products of investigations involving service equipment. Although none of them saw wide operational use, several of the ideas will undoubtedly be incorporated in future developments. It is not appropriate to give a complete list in this place, but an indication of their range can be gathered from the following examples: safety clamps for replacing the cat's cradle of cables connecting the flier to his plane with a single cord, a self-rescue apparatus for submerged aircraft, an oxygen warning panel for bombers, a locking mask connector, and an automatic parachute opener.

One item of oxygen equipment that was unsatisfactory was the stand-by or emergency supply system. Small low-pressure "walkabout" bottles were available for bomber crews when they moved from their fixed stations, but they were both too bulky and of too small capacity to fill the need adequately. The Committee on Medical Research and National Defense Research Committee jointly undertook the development of two promising types of expendable chemical oxygen units, which were unfortunately only ready for service testing at the close of the war. In each the oxygen was stored in chemical form in a light tin container. One utilized a chlorate "candle," which burned for half an hour, yielding 5 l. of oxygen per minute. The other was a rebreather chemical demand system utilizing potassium tetroxide,

which lasted for about two hours. They each weighed about 3 pounds and were extremely compact. In terms of both weight and volume for a given length of service, they were many times as efficient as the tank system they were designed to replace.

TRAINING PROGRAM FOR AIR CREWS

Minor but tangible contributions made by the Office of Scientific Research and Development were in the field of crew training. With the advent of massed bombing raids at high altitudes, a most formidable educational problem was superimposed on the gigantic task of producing the planes. The men had to be trained to fly them. Extensive cadet training courses were quickly organized by both Army and Navy, with the assistance of Committee on Medical Research personnel; about a hundred altitude chambers were erected for demonstrating the effects of anoxia to prospective crews, and several hundred aviation physiologists were recruited and trained for instructional duties. These instructors and occasional research workers, scattered in remote localities all over the world, needed all the authoritative physiological data pertinent to aviation that they could obtain. The Handbook of Respiratory Data in Aviation, referred to above, was distributed to these men, supplementing the material from their service headquarters, and proved to have wide usefulness.

A second contribution was the oximeter, a teaching aid for altitude chambers. This device, which measures the level of oxygen in the blood photoelectrically and presents the information directly on a large scale, gave dramatic indication to cadets of changes taking place in their bodies preceding anoxic collapse, and emphasized for them the necessity for using their oxygen equipment intelligently. They were installed in some sixty altitude chambers of the Army and Navy.

A third contribution to the high-altitude training program was the occasional use of the research laboratories for special courses of training in particular operations. Sixty Army fighter pilots were given training in emergency parachute descents in the Welfare Island chamber, the Yale University chamber was used in training flights for over two hundred pilots engaged in government agencies, and the University of Pennsylvania chamber was employed to demonstrate the effects of anoxia on vision to the aviation physiologists assigned to the Night-Vision Training Program.

RESPIRATORY PHYSIOLOGY AND ANOXIA

Perhaps the greatest lasting contribution of the wartime activities of the Office of Scientific Research and Development consists in the consolidation of knowledge. This is nowhere better illustrated than in the field of respira-

tory physiology. The development here is analogous to that wrought by Henderson in blood biochemistry a generation ago.

This consolidation represented an integration of the discoveries of Bert, Haldane, and other physiologists, put into mathematical and graphical form. The principal contributors to the newer synthesis have been Berkson, Boothby, Bateman, Gray, Fenn, and Brink. From them, we have learned how to deal quantitatively and simultaneously with the several factors that determine respiratory gas exchange (namely, the total barometric pressure, the composition of the inspired gas, the alveolar tensions of oxygen and carbon dioxide, and the alveolar respiratory quotient), and we have learned how these change with the alveolar ventilation rate. The principal stimulus for this synthesis came from the practical problems associated with flying, such as design of oxygen regulators and estimations of the efficacy of emergency breathing procedures. The results, however, are not limited to aviation, although they have found their most interesting application to date in pressure breathing and in hyperventilation.

The so-called "alveolar equation" that defines this relationship may be expressed in many forms; three of the most useful, in ascending order of generality, are as follows:

(1) Where pure oxygen is breathed; $FO_2 = 1$; $FCO_2 = 0$

$$pO_2 = B - 47 - pCO_2$$

(2) Where oxygen-nitrogen mixtures are breathed; $FCO_2 = 0$

$$pO_2 = (B - 47)FO_2 - pCO_2 \frac{1 - FO_2(1 - RQ)}{RQ}$$

(3) Where mixtures of oxygen, nitrogen, and carbon dioxide are breathed:

$$pO_2 = \frac{(B - 47)(RQ \times FO_2 + FCO_2) - pCO_2(1 - FO_2(1-RQ))}{RQ + FCO_2(1-RQ)}$$

Notation:

pO_2 = alveolar oxygen tension in millimeters of mercury
pCO_2 = alveolar carbon dioxide tension in millimeters of mercury
B = barometric pressure in millimeters of mercury
47 = vapor pressure of water at body temperature, in millimeters of mercury
FO_2 = fraction of oxygen in inspired gas
FCO_2 = fraction of carbon dioxide in inspired gas
RQ = respiratory quotient (alveolar)

Equation 2 is the most widely used. These equations (and the supplementary ones dealing with ventilation rates) can be illustrated graphically by charts in which alveolar pCO_2 is plotted against alveolar pO_2 (Fig. 38). These charts bring out the essential physiological relationship in a clear and quantitative way and have been extremely useful in teaching and research.

The alveolar equation has been used to calculate the proportions in which oxygen should be added to air in order to maintain sea-level conditions at altitude, and the proportions in which the oxygen fraction should be reduced
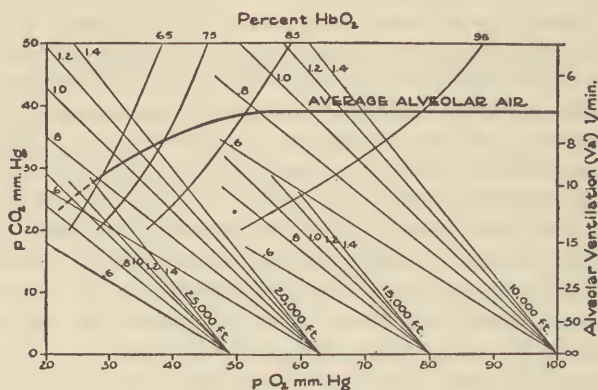
FIGURE 38. *Chart of alveolar gas compositions.* (From Fenn.)

in order to produce any desired equivalent altitude (Fig. 39). Many experiments and much friendly debate have forced a careful analysis of the assumptions involved in the alveolar equation, and have brought out in clear relief both its strength and its limitations. These have been presented in
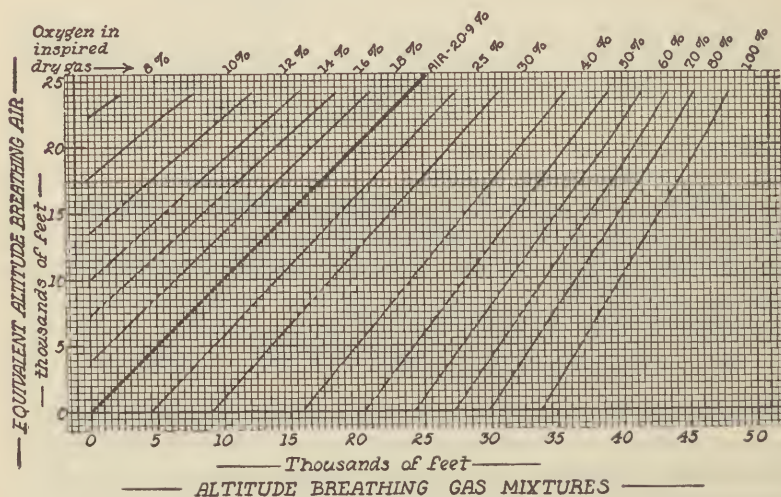
FIGURE 39. *Equivalent altitudes breathing gas mixtures.*

the authoritative version prepared for the Handbook of Respiratory Data in Aviation.

The upshot of the discussions has been the conclusion that the alveolar equation can properly be used to calculate the oxygen fractions required to maintain sea-level conditions at altitude, as required in the design of oxygen equipment. It is at least as accurate as the alternative "tracheal standard" proposed by Boothby, which ignores the gas exchange taking place in the lungs, and from which it differs numerically by less than 5 per cent. Under anoxic conditions, however, the alveolar equation, although still very useful, must be employed with caution, lest unwarranted assumptions of constancy in the alveolar pCO_2 or in the respiratory quotient slip into the figures. For calculating equivalent altitudes under more or less steady conditions, its predictions have agreed closely with experimental determinations of arterial oxygen saturation and of performance tests (Fig. 40).
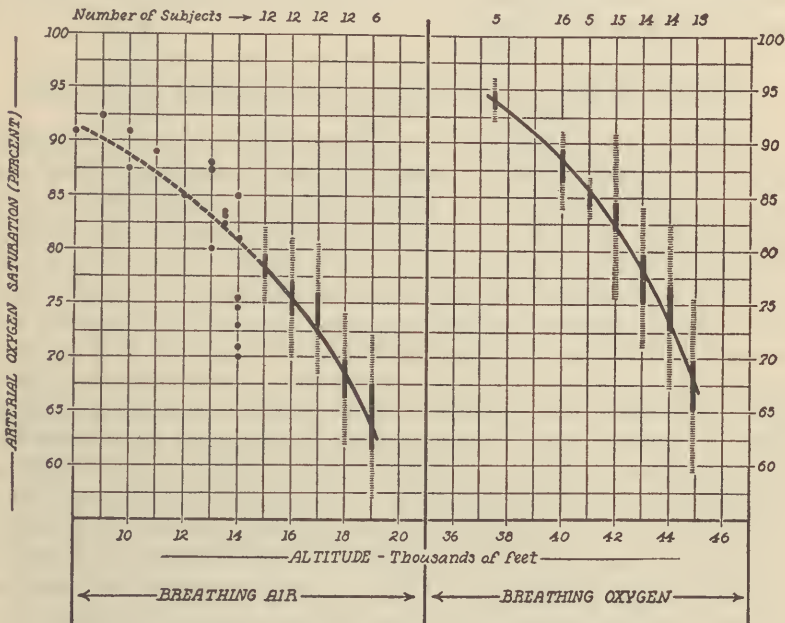
FIGURE 40. *Arterial oxygen saturation at altitude.*

An important application of the alveolar equation (especially in Fenn's chart form) has been the estimation of differences between steady and non-steady state of respiratory exchange. It has been the essential tool in assaying the relative benefits derived from pressure and from hyperventilation in

pressure-breathing and voluntary-breathing procedures. These will be discussed in the next section.

The relative dangers of anoxia and acapnia have been much discussed, but until recently no quantitative information concerning their separate and combined effects has been available. This is a matter of considerable practical importance, since the balance between the two can be altered by changing the ventilation rate. Fenn and his colleagues have recently administered a contrast-discrimination test and a hand-steadiness test to subjects whose alveolar oxygen and carbon dioxide tensions were maintained at various desired tensions. The results show that anoxia and acapnia are additive in their effect. For any given altitude, there is an optimum alveolar pCO_2 level for best performance, which can be attained by proper adjustment of the ventilation. This optimum is close to that reached naturally by subjects whose altitude tolerance is good. Inexperienced subjects, who ventilate either too little or too much, will find their performance impaired — the one from anoxia, the other from acapnia.

PRESSURE BREATHING AND HYPERVENTILATION

Of all the methods that have been suggested for increasing man's ceiling, pressure breathing received by far the greatest attention during the later years of the war. This is a device for raising the altitude tolerance by increasing the pressure of the inhaled gas a few millimeters of mercury above that of the ambient barometric pressure. The practical possibilities of pressure breathing were first explored in the Aeromedical Laboratory at Wright Field. There special pressure-breathing equipment was rapidly developed, and several special high-altitude photo-reconnaissance squadrons were equipped and trained for its use. Although the number of military flights actually flown at altitudes above 38,000 feet, where pressure breathing is required, was very small, the potential importance of this equipment was considerable, since it was not available to either Germany or Japan. The entire program of equipment development, including both regulators and masks, was under the direction of the Army Air Forces; the physiology of pressure breathing, on the other hand, was studied extensively under OSRD(CMR) auspices as well as in service laboratories. While the first overenthusiastic claims for pressure breathing have had to be toned down somewhat, the balance sheet drawn up at the end of September 1945 for the Committee on Medical Research by four of its leading investigators in the field leaves no doubt that the method has a real value.

Three principal contributions were made by our investigators to the knowledge of pressure breathing: the elucidation of the actual gain in altitude tolerance that could be obtained with it, the relative parts played by pressure and by hyperventilation in this gain (especially during intermit-

tent pressure breathing), and finally an analysis of the physiological limita-
tions of the method. When pressure is applied to the lungs, they tend to fill
up. The respiratory reflexes are reversed; expiration is active and inspira-
tion is passive. This may change the breathing pattern and the total amount
of air breathed per minute. A large number of careful experiments made in
many different laboratories have now shown, however, that if not over 8
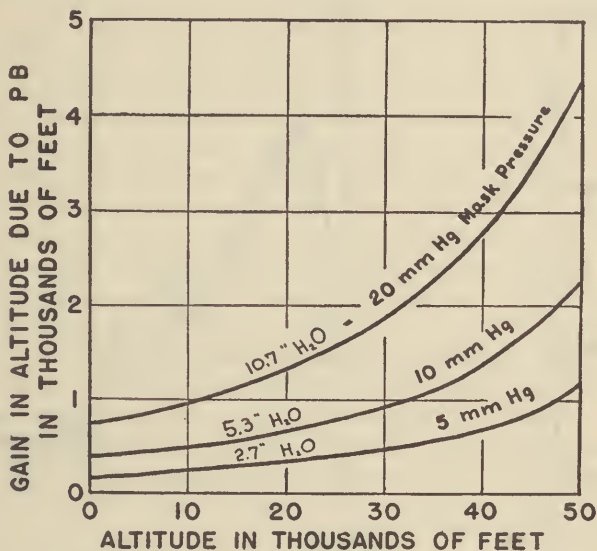
FIGURE 41. *Gain in altitude due to pressure breathing.*

inches of water is continuously applied to the breathing pressure, the *average*
altitude ceiling is increased by an amount that corresponds closely to the
applied pressure. Simple physical principles may therefore be applied in
calculating the gain, and a maximum boost of 2500 to 3000 feet can be
obtained at an altitude of about 40,000 feet (Fig. 41). This conclusion is
based both on psychomotor performance tests and on measurements of
arterial blood saturation. However, wide individual differences exist, owing
to the varying responses of respiration and of circulation. When pressure is
first applied there is often a large transient rise in saturation, especially in
subjects who have not had much previous experience. This is due to a tem-
porary hyperventilation, which blows off carbon dioxide and increases the
alveolar oxygen tension by an equal amount (Fig. 42).

If the pressure is intermittently applied, as in some types of regulators,
such as the GE pneumolator and the Bennett valve, there is a much more
pronounced tendency to hyperventilate, which may lead to serious acapnia.

The method is therefore easier but more dangerous. Since the pressure is only applied for a fraction of the respiratory cycle, the peak pressures required to maintain a given average pressure are higher with the intermit-
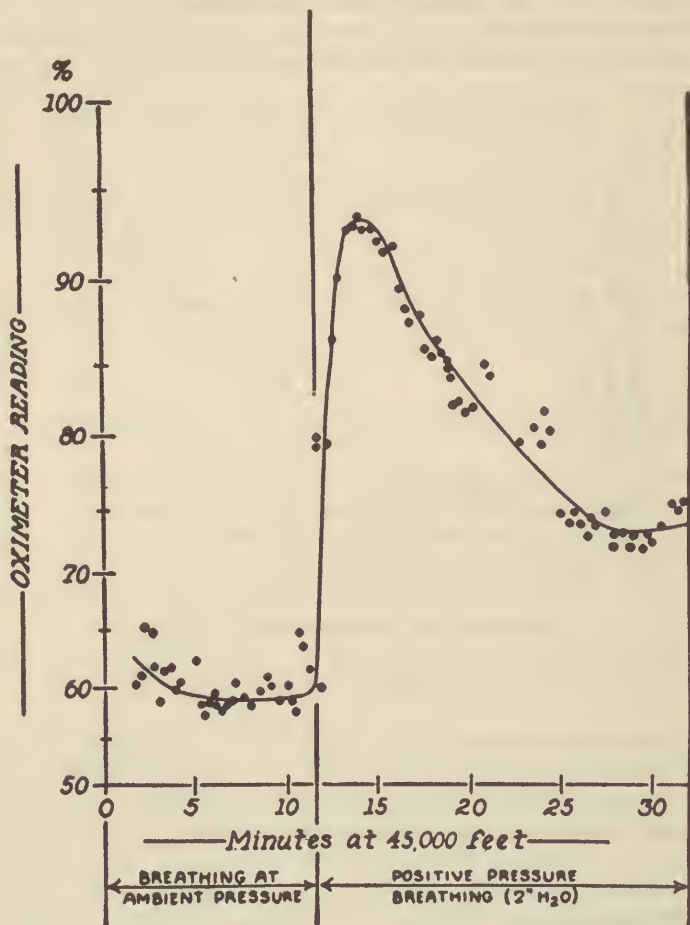
FIGURE 42. *Combined effect of pressure and increased ventilation on arterial oxygen saturation.*

tent method, which reduces somewhat its added convenience. The combined effects of pressure and of overventilation have been analyzed with the aid of the alveolar equation, from which the probable alveolar levels of oxygen and carbon dioxide can be predicted, and from these the approximate performance can be estimated. Agreement has been good.

The limits to the pressure that one can tolerate depend on the elastic properties of the lung and chest and on the necessity of venous return to the heart. Analysis of the pressure-volume diagram of the lung has shown that about half the pressure is absorbed by the elasticity of the lung, the rest being passed on to the pleural cavity, requiring an increase in venous pressure. When this adjustment fails, collapse occurs. Syncope occurs more frequently at altitude with pressure breathing than without, owing to pooling of blood in the veins. Consistent with this is the finding that cardiac output is somewhat reduced, as shown both by the direct Fick method and by the ballistocardiograph. Distension of the chest wall can be reduced by counter-pressure, and pooling of the blood by G-suits. With such devices "ascents" to 58,000 feet have been made at the University of Southern California for brief periods, using a pressure of 35 inches of water.

Various voluntary breathing procedures have been suggested from time to time for increasing altitude tolerance during emergencies, when oxygen is not available. Startling increases in arterial oxygen saturation were reported for the procedure introduced by Lieutenant Commoner of the Navy, known as voluntary pressure breathing, or VPB. This consisted of a forced expiratory effort with the glottis closed during each respiratory cycle. The same type of analysis that had proved successful in elucidating the factors involved in pressure breathing at extreme altitudes was applied here for the air-breathing conditions. The results were confirmed, but it was shown that much the most important factor responsible for the gain in altitude is the hyperventilation that occurs. A number of variants of the procedure have been investigated, but it now appears that the easiest of all procedures (simple hyperventilation) is as effective in maintaining consciousness at 25,000 feet as is any other method.

The problems of pressure breathing provided a challenge to physiologists. It was a magnificent opportunity for them to investigate processes with which they were familiar under new and unusual stresses, and to determine the over-all results on the organism. As a result of their studies, it is now generally agreed that there are no mysterious or unexplained factors involved in pressure breathing.

OTHER MEANS FOR ALTERING ALTITUDE TOLERANCE

In any struggle in which the contestants are well matched, a small advantage possessed by one of them may have a practical effect out of all proportion to its magnitude. By raising the effective ceiling of one's own fliers a thousand feet above that of one's adversaries, a situation might suddenly arise in which one could enormously extend one's range or reduce one's losses. We were in duty bound, therefore, to investigate every prospect of increasing a

flier's altitude tolerance, however small the chance of success. Even though we knew that ultimately the engineer with his pressure cabin would be able to outstrip the physiologist with his ameliorative adaptations, we could not evade this responsibility. In addition to pressure breathing and hyperventilation in their various forms, a number of other methods for increasing altitude tolerance were suggested, some of them fantastic. Most of them were given at least an opportunity of showing what they could do in altitude-chamber experiments.

These methods were of three functional types: those that affected tissue metabolism, such as nicotinic acid amide, adrenocortical hormone, and methylene blue; those that presented a novel manner of administering oxygen to the body, such as direct intravenous injection and ingestion of organic peroxides; and those that primarily affected the transport systems of respiration and circulation, such as ammonium chloride, high-carbohydrate diets, and carbon dioxide inhalation. In performance tests at air-breathing altitudes the first two categories gave estimated improvements ranging from zero to 2000 or 3000 feet; in the oxygen-breathing high-altitude range, the gain would have been about half as much (Fig. 39). Since pressure breathing could do more, these small altitude gains did not justify the use of any of these methods in actual operation.

The most persistent claimants for adoption, however, were those of the third category. These depended primarily, at least in theory, on their effect in improving gas exchange in the lungs, although other complicating factors were also active. They were tested for the most part at air-breathing altitudes, but it could be predicted from the alveolar equation that the beneficial effects would be much reduced or entirely eliminated when they were applied to the high-level oxygen-breathing range. Their proposed tactical use, therefore, was primarily restricted to medium-altitude missions where oxygen equipment either was not available or was not functioning in emergencies. Ammonium chloride, the most readily available nontoxic acid, was known to produce hyperpnea, thereby reducing the carbon dioxide tension and raising the oxygen tension in the alveoli. As shown both by pursuit meter tests and by studies of blood chemistry, ingestion of 20 or 30 gm. of this substance did produce an increase in altitude tolerance up to 5000 feet for several days, but voluntary hyperventilation, without the drug, can do about the same.

If the respiratory quotient is increased by a high-carbohydrate diet, there should also be a gain in altitude tolerance for air-breathers, although not for those breathing oxygen (Fig. 38). This was pointed out before the war. The question was studied extensively by several groups during the war; the gain was found to be a real one but was not more than about 2000 feet.

Inhalation of carbon dioxide as a means of gaining altitude was frequently recommended. At oxygen-breathing altitudes, the measure would seem to defeat itself, causing only increased ventilatory effort, for every

added millimeter of carbon dioxide tension reduces the oxygen tension by the same amount. The weight of experimental evidence is in this direction, in spite of some contrary evidence. At air-breathing altitudes, the situation is more complicated owing to the possibility of nitrogen dilution, but there is little question that addition of a suitable amount of carbon dioxide to inspired air will increase both the alveolar oxygen and carbon dioxide tensions. However, it would be more advantageous to add an equal amount of oxygen, which is much easier and safer. The suggestion, therefore, has no practical utility.

A considerable amount of work was done in measuring the deleterious effects of substances that might be either inhaled or ingested by aircraft crews. Much the most important of these was carbon monoxide, long recognized as a military hazard because of its large concentrations in the exhaust gases of internal engines and in gun fumes. Our investigations included field surveys of carbon monoxide prevalence, studies of its rate of uptake and elimination by man, and various studies of its effect on man. Owing largely to the extensive work carried on by the services themselves and to their constant vigilance, carbon monoxide was not a serious problem during the war, except in the minds of fliers. Numerous tests were also made of the effect on altitude tolerance of many drugs used in medication, such as quinacrine, the sulfonamide drugs, ephedrine, amphetamine, and so forth. These studies added little to our understanding of the body's processes, but they provided essential data on which operational standards and regulations were based.

INSTRUMENTS

It could be argued that our most important concrete contribution to high-altitude operation that made itself felt during the war was the development of numerous instruments for measuring more conveniently or more quickly quantities frequently required in laboratories and training stations and during field operations. For the most part these devices did not achieve accuracies surpassing those of the more cumbersome classical methods, but by their adaptability to awkward conditions of space and time they made possible the collection of useful data that could not otherwise have been obtained. As was the case in the other activities of the Office of Scientific Research and Development, the relative contributions of civilian and military agencies were so intermingled that an accurate assessment of credit is quite impossible; in fact, the better the collaboration the more difficult it is to separate the elements responsible for the success of the venture. Usually, the original request for a given device came from a service laboratory or field station. The inevitable compromises in accuracy, simplicity of operation, ruggedness, and so forth were worked out jointly as development proceeded. If a device promised well for a particular service activity, it was often taken over by the

interested branch, or parallel developments were carried on by both civilian and military groups.

Several advances of importance were made in technics for analyzing respired gases. The simplest and most strikingly successful was the quick gas analyzer devised by Scholänder (Fig. 43), which was so simple and convenient to operate that it was widely used in testing oxygen masks for leaks in the laboratory, in the field, and even in the air. Its accuracy (from 0.3 to about 1.0 per cent) is adequate for most physiological test work. The ingenious elimination of stopcocks and the use of an ordinary syringe for collection and measurement of the gas samples endeared it to those accustomed to make analyses with the more accurate but cumbersome Haldane apparatus. This device scored over its predecessors, not because it could do more than they could but because it could perform the task more simply.

In respiratory physiology and in the development of oxygen equipment, there was a great need for continuous gas analyzers, and this need was met by the development of a whole battery of devices. Among these were the Pauling oxygen meter, the Pfund infrared analyzer, primarily useful for carbon dioxide and carbon monoxide, the Berg thermal-conductivity meter for oxygen and carbon dioxide, and the Lilly nitrogen meter. The Pauling and Pfund meters became available on a commercial scale during the war. They were widely used in several branches of pure and applied research and testing. The Pauling meter depends for its operation on the paramagnetic properties of oxygen, which distinguish it from other respiratory gases. It was calibrated in manufacture to give direct readings of oxygen partial pressure independent of the presence of other gases or of changes in total pressure, which made it a very useful tool for work in altitude chambers.

The Pfund instrument utilized the strong infrared absorption bands of carbon monoxide and carbon dioxide. It is extraordinarily sensitive, and can detect and measure minute quantities of both these gases. It would have found much wider employment in aviation had it been more readily available.

Berg's analyzer for oxygen and carbon dioxide is based on the thermal conductivity of these gases, as measured by the cooling of hot wires. It follows the basic construction of earlier devices, but adapts them to the ranges required in aviation medicine. It provides analyses at fifteen-second intervals.

All the above devices are relatively slow, providing analyses of gas collected over periods ranging from five seconds to several minutes. They are therefore not suitable for analyzing the changes in gas composition occurring during a single respiratory cycle. An entirely novel analytical procedure is utilized in the nitrogen meter developed by Lilly (Fig. 44). Gas is drawn continuously from the point of analysis through a minute aperture, usually in the tip of a needle placed in the gas stream, and is sucked at high speed to a specially
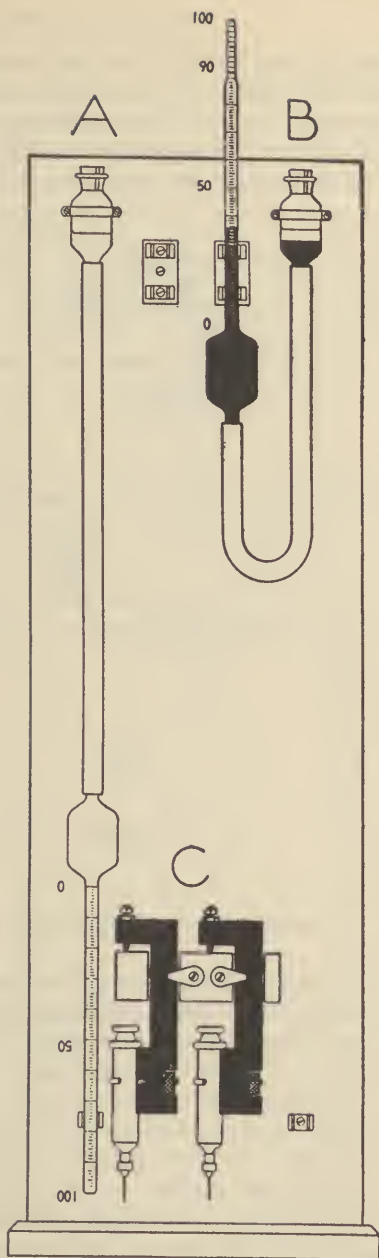
FIGURE 43. *The Scholänder quick gas analyzer.*

constructed gas-discharge tube. The light emitted is measured by a filter-photocell combination. By isolation of the correct spectral reion, the response is determined almost entirely by the nitrogen present, being independent of the presence of other respiratory gases. With a collection tube 2 feet in length, time lags as short as 0.02 second can be obtained, making it
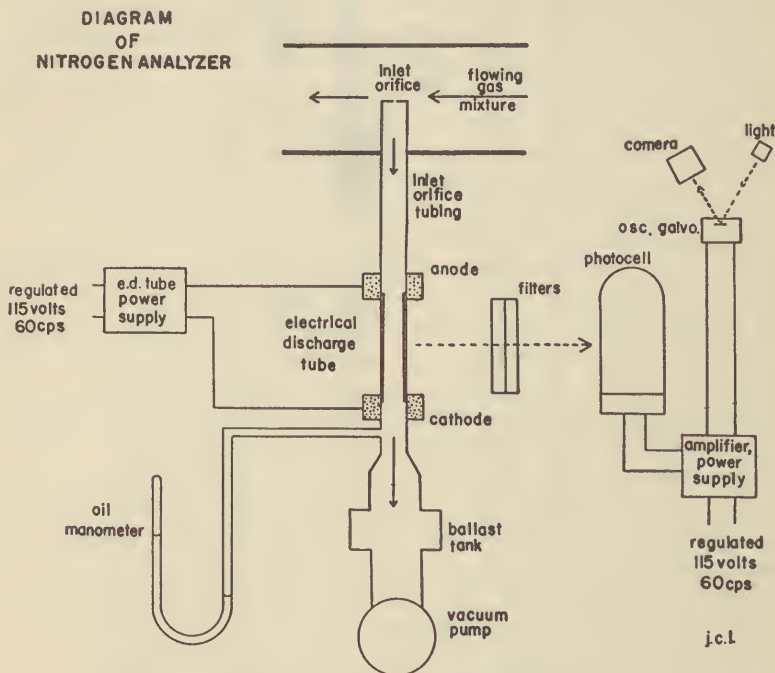
FIGURE 44. *Diagram of the Lilly nitrogen meter.*

possible to follow the composition of gas throughout the respiratory cycle. A number of these devices have been used in laboratories of aviation medicine to study the mechanism of gas exchange in the lung. They give promise of future value in the analysis of respiratory impairment.

Before the war, no practical means was available for following continuously the oxygen level in the blood. The photoelectric oximeter, to which reference has been made above, was developed for this purpose and has been widely used in cadet training and in research. With this device, the color of the fully flushed ear is measured by a small unit that slips over its shell. It has been possible to calibrate the instrument directly in percentage of oxygen saturation of the arterial blood, utilizing a two-color principle that automatically compensates for variations in the amount of blood in the ear. The

device is accurate to about 4 per cent and requires no calibrating punctures.

In addition to its use in Army and Navy altitude training units, the oximeter was used in most of the aviation research laboratories of the country, both for physiological work and for testing the adequacy of oxygen equipment. A simplified form, the oxygen want indicator, was developed for use in aircraft, the ear unit being mounted in the helmet and integral with the telephone receiver, with the indicating unit, about the size of an altimeter, on the instrument board. Anoxia was not a serious enough problem to warrant the adoption of this device for routine use, although it was employed in some flight research work. The oximeter gives promise of being useful in peacetime, for the control of anesthesia, the diagnosis of respiratory disease, physiological instruction in medical schools, and the regulation of oxygen therapy.

Another device for the analysis of blood gases, which was widely used, was the Scholänder-Roughton syringe analyzer. Although originally introduced before the war, it was further developed under OSRD(CMR) contract and was widely used for carbon monoxide and oxygen determinations of small blood samples. The accuracy was a little less than that of a Van Slyke apparatus, but the entire apparatus with its equipment could be carried in a cigar box, and it required no laboratory fixtures.

A considerable number of experimental methods were developed for purposes of particular researches, of which several have found application elsewhere. Among them were two distinct types of rapid respiratory flowmeters. One, produced at Harvard University, depended on the displacement in the air stream of a fine quartz fiber. With it has been obtained the most extensive and authoritative set of data on respiratory flows and respiratory patterns now available for calculating design requirements of respiratory equipment. The other method, worked out at the University of Pennsylvania, utilized a fiberglass resistance unit and an electrical capacitance manometer. Manometers of this type, which can be made extremely sensitive and extremely fast, have been supplied for use in many researches in related problems of aviation medicine. They have been employed to record mask pressures (in addition to mask flows), and cabin pressures during explosive decompression, as well as for accurate tracings of blood-pressure changes. Other methods with which new physiological information has been gained include a technic for the measurement of cerebral blood flow in man, utilizing nitrous oxide, and a new method for the continuous sampling and analysis of alveolar gases. This list is far from complete.

Both the physiological technics and the instrumental facilities of contractors were frequently called on for tests of apparatus and procedure. Thus, the Pacific Coast groups at Berkeley and Los Angeles co-operated extensively with the services in experimental high-altitude flights in airplanes; the low-temperature characteristics of regulators were studied at Yale; pressure-

breathing regulators, both continuous and intermittent, and pneumatic balance resuscitators were investigated at the Mayo Aeromedical Unit and at various universities.

CONCLUSIONS

One cannot close this account of the oxygen activities of the Committee on Medical Research without attempting to gauge the trends in the field as the war closed and to assess the value of the work that has been done. The fleets of B–29 bombers striking daily at Japan in the last year of the war gave dramatic evidence that the pressure-cabin military airplane was an actual fact. For normal operation, this meant either that oxygen could be dispensed with or, in the case of very high-altitude planes, that standard oxygen equipment would be adequate. The main emphasis in the field therefore shifted to problems of emergency, of exploded cabins, of parachute drops from extreme altitudes, and of procedures that would prolong life for a few precious seconds until a safe environment could be reached. Here, conditions are rugged and human experimentation hazardous. The measure of success is not the maintenance of a high level of performance, but bare survival.

Early in the war, it had been shown that parachute descents from 40,000 feet could be made without oxygen equipment. New emergency pressure suits were developed to give sufficient protection for brief ascents to about 60,000 feet. Here arose a new demand for a closer liaison between physiologist and engineer, reminiscent of that which proved so fruitful in the early development of the diluter-demand regulator. A whole group of new devices is now required to increase one's chance of survival: ejection seats, body-worn oxygen equipment, inflating cuffs, automatic parachute ripcords, and others. For the design of each of these, we must know the strains to which the body can be subjected, not singly or in succession but with their combined impact. Here, departmentalization breaks down and a larger synthesis is needed. The fields of anoxia, explosive decompression, decompression sickness, extreme cold, and high accelerations are all involved and must be welded into one. By such devices as these and by suitable procedure for their effective use we may tempt man to fight a few miles higher in the stratosphere, until at last the guided missile replaces the human being in the machine, and a chapter of aviation physiology will be closed.

In any summary of wartime research activities, the interest has now naturally shifted to the byproducts that may have lasting peacetime value. He would be rash who would predict with confidence, but a few suggestions may be made. As a result of the intensive study of the mechanism of respiratory processes stimulated by aviation's need, there is now a much wider understanding and interest in these problems than there was before the war. The application of this knowledge to respiratory abnormalities of disease, in

poliomyelitis, emphysema, tuberculosis, and pneumonia, is widespread and is growing. At least one new theory of respiration owes its existence to the study of anoxia at altitude. The technics and instruments developed for aviation are already widely employed. Few researches on respiratory problems now fail to make use somewhere of a gas-analysis device, or of an instrument for measuring blood gases, or of a demand valve, developed by the Office of Scientific Research and Development for war purposes. But more important than these, the wartime researches provided a background of understanding on which future applications could be more firmly based, a reservoir of ideas and of minds awake to the problems of anoxia, and a spirit of joint enterprise in new tasks whose challenge carries over into the tasks ahead.

CHAPTER XXV

ALTITUDE DECOMPRESSION SICKNESS

JOHN F. FULTON

IN SEPTEMBER 1939, at the beginning of World War II, it was dimly appreciated that exposure of flying personnel to extremes of altitude might precipitate symptoms similar to those experienced by divers and caisson workers when decompressed too rapidly from ambient pressures greater than the ordinary atmosphere. Armstrong, in his *Principles and Practice of Aviation Medicine,* published in November 1939, popularized the belief that the so-called "bends" symptoms experienced at extremes of altitude are due to the formation of nitrogen bubbles within the body, presumably in certain of the smaller blood vessels (hence the term "aeroembolism," which we now know to be misleading). Armstrong's emphasis on the similarity between altitude pains and the bends pains of divers gave support to the conviction that the problem should be analyzed in terms of rates of nitrogen elimination, as in the case of caisson workers' bends. Armstrong proved that bubbles — presumably of nitrogen — could, in fact, be demonstrated in the veins of experimental rabbits exposed to altitudes of 38,000 feet. He was not able, however, to demonstrate tissue bubbles, but on theoretical grounds he was prepared to believe that bubble formation might occur both in the blood vessels and in the tissues.

Off and on since the time of Paul Bert, human beings have been exposed in low-pressure chambers, but the first acute case of altitude bends of which I have found record is that described by Boothby and Lovelace in the *Journal of Aviation Medicine* in December 1938. They record that Dr. Heim, when acting as a subject during an altitude run to 35,000 feet, suddenly became paralyzed from the waist down at a time when he was breathing 100 per cent oxygen and was exhibiting none of the usual symptoms of anoxia.[1]

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[1] The first one to consider the possibility of altitude bends was the late Yandell Henderson. Writing in 1917 on the effects of altitude in aviators (*Aviation and Aeronautical Engineering,* 1917, 2:145–147), Henderson, after describing caisson disease, writes: "In order for bubbles to be formed it is essential, however, that the pressure with which the tissues are in equilibrium should be lowered more than half its absolute amount in a few minutes." He continues: "In the present state of the art of flying it is scarcely possible for an aviator to rise to a height of 20,000 feet, where the barometer would be about half of that at sea level, in a period sufficiently short to allow bubbles of nitrogen to form in this way. The disorders from which aviators suffer therefore are of a different class from those to which workers in compressed air

His symptoms disappeared on returning to sea-level pressure. As high-altitude studies became more numerous in the early months of 1939, many more instances of acute pain at altitude, often associated with other neurologic symptoms and often developing with dramatic suddenness, were placed on record, and it immediately became evident to observers in Germany, Italy, Canada, Great Britain, and this country that high-altitude bombing operations would involve, in addition to the hazards of anoxia, another danger of a major character; namely, that of decompression sickness. The terminology used in the early years of the war lacked precision; some writers, following Armstrong, used "aeroembolism," others "aeroemphysema," and the term "bends," borrowed from the caisson literature, was often used interchangeably with these terms. It soon became evident, however, that the syndrome of altitude illness involved more than pain and prostration, for there were many premonitory signs and symptoms, as well as serious post-flight reactions involving both the nervous and vascular systems, and for this reason it has seemed desirable to designate the total syndrome as "altitude decompression sickness" in order to distinguish it from "high-pressure decompression sickness," which has distinctive clinical characteristics.

In June 1940, Drs. Bryan H. C. Matthews and Arnold C. Carmichael, on behalf of the British Flying Personnel Research Committee, requested the group in the physiological laboratory at Yale University to undertake experiments on possible ways and means of preventing symptoms of decompression sickness in monkeys and chimpanzees — this with a view to working out denitrogenation procedures that might serve to protect flying personnel from disabilities arising when at altitude. The Office of Scientific Research and Development had not then been formed, but when the Committee on Aviation Medicine had been appointed by the Division of Medical Sciences of the National Research Council in September 1940, the Committee began at once to turn its attention to the problem of decompression sickness, and on October 31 it recommended that a full-time man be engaged to investigate the problem. In the report made during November 1940, after a trip of the Committee on Aviation Medicine to various air bases and to Sir Frederick Banting's aeromedical research laboratory at Toronto, the Committee made the following recommendation, which serves clearly to indicate the state of knowledge of the subject at that time.

Air embolism. The reports from England indicate fairly extensive disability among fliers passing rapidly from sea level to high altitudes. It is a problem under investigation at Toronto, in the experimental diving unit in Washington in the National Institute of Health, and in England. It may be pointed out that

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are exposed." Henderson thus foresaw the possibility of altitude bends, but he insisted, quite rightly, that if aircraft did not fly above 20,000 feet and since they required considerable time to achieve that altitude, decompression sickness would probably not be encountered by flying personnel.

at the present time the exact pathogenesis of the "bends" is not known either in decompression from high atmospheric pressures or under the present new group of circumstances; nor have we satisfactory methods for preventing and remedying these distressing occurrences. The Committee feels that the problem requires immediate experimental attention, and regards the diving unit in charge of Lieutenant A. R. Behnke in Washington as peculiarly suitable for experimentation in the United States on account of the qualifications of the subjects available to Lieutenant Behnke, together with his great experience in examining the problem under conditions of high atmospheric pressure. Dr. Behnke's group should be in close touch with the valuable observations being made in Toronto and at Wright Field.

Several weeks after the Committee on Aviation Medicine had made the above recommendation, Lieutenant Behnke, reporting from the Experimental Diving Unit at the Washington Navy Yard, found that his experienced deep-sea divers developed the bends syndrome following rapid ascent to simulated altitudes and that given persons tended, at altitude, to develop bends pains at the same sites at which they had experienced pain on emerging from a diving operation. He proved, as had Lovelace the year before, that elimination of nitrogen by oxygen breathing prior to flight protected his divers from decompression sickness; indeed, they were able to ascend from ground level at the rate of 5000 feet per minute to an altitude of 37,000 feet and remain there, symptom-free, for four hours. To reduce the body's nitrogen, however, to a level that afforded complete protection requires at least three hours of oxygen breathing prior to flight, and since this would seldom prove feasible for flying personnel, other ways of affording protection were studied by Behnke and by various groups presently to be sponsored by the Committee on Aviation Medicine and the Office of Scientific Research and Development.

Since flying personnel in large numbers soon began to be indoctrinated in the use of oxygen equipment in low-pressure chambers, the hazards of decompression sickness (and of the debilitating and sometimes even fatal reactions that follow an acute bends episode at altitude) were quickly impressed on our flight surgeons, and also on those responsible for conducting investigative studies in the field. Requests from the armed forces for further information had become so insistent during 1941 that the Committee on Aviation Medicine, which had already endorsed through the Office of Scientific Research and Development six projects in which decompression sickness was to be studied, recommended the formation of a separate Subcommittee on Decompression Sickness, which would concern itself with the study of this problem, using the broadest possible approach. After discussion, Dr. Weed as Chairman of the Division of Medical Sciences phrased the Committee's mandate as follows:

The Committee will be expected to advise the medical services of the Army and Navy Air Corps on problems of aeroembolism, with particular reference to

its nature and means of prevention. From the military standpoint aeroembolism has become somewhat less of a problem through disclosure of the fact that a large proportion of young male adults are relatively insusceptible to its painful complications (bends); if high-altitude squadrons are preselected on the basis of their resistance to bends, operational difficulties in the high-altitude range can be minimized. Even though less serious from the military point of view than was at first supposed, aeroembolism remains a scientific problem of deep and challenging interest; and since preselection of large numbers of air force personnel is time-consuming, and since such testing, although possible, would overtax existing decompression-chamber facilities, any procedure or agent which will minimize the dangers arising from aeroembolism will be welcomed by the Services.

The Committee therefore will be expected to study the question from every angle, including the basic physical chemistry of bubble formation in tissues and protein solutions, and to analyze conditions which favor or hinder their appearance; and from the immediate operational standpoint we must examine the efficacy, for the prevention of bends, of preoxygenation, exercise, and the use of helium and other gases, as well as possible prophylactic effects of drugs and special diets. Pressure devices designed to protect flying personnel must also be looked into, including the various pressure suits, pressurized cockpits, and the many important physiological problems arising from recent work on pressure-cabin aircraft.

The Subcommittee on Decompression Sickness, which was thus appointed on April 13, 1942, had nineteen formal meetings prior to its dissolution on June 30, 1946 — eighteen at the National Research Council in Washington and one at the Donner Research Laboratories in Berkeley, California. During this period it sponsored, through the Committee on Aviation Medicine and the Committee on Medical Research, fourteen separate contracts for study of specific phases of decompression sickness, and it also endorsed four general contracts already sponsored by the Committee on Aviation Medicine in which studies on decompression sickness were being undertaken. The total expenditure involved in the Office of Scientific Research and Development contracts in question during the four-year period of the Subcommittee's operation amounted to $1,480,000.

The reports of studies carried out under the Subcommittee's contracts were issued in the first instance in the Committee on Aviation Medicine series. Of the five hundred and sixty reports issued to date, one hundred and eighty have had to do with decompression sickness or the closely related problem of explosive decompression. Since the Subcommittee was strong in its conviction that the information thus accumulated should be integrated and presented in the form of a continuous narrative, the Division of Medical Sciences has recommended that the Subcommittee edit and publish a detailed monograph on the general subject of altitude-decompression sickness. The monograph is now in press and will presently be published. In addition, material contained in many of the Committee on Aviation Medicine reports has been released for publication in scientific journals.

Problems studied by the Subcommittee group themselves into two primary categories: theoretical studies and practical applications. Under the first category the Subcommittee took up four principal problems: physical factors and bubble formation in fluids and in animal tissue, including histologic studies; bodily and environmental factors affecting the incidence of decompression sickness in man; studies in preoxygenation and elimination rates of inert gases from the body; and deformation pressure. Under practical applications the Subcommittee was asked to make recommendations concerning preselection testing procedure for elimination of bends-susceptible subjects, application of preoxygenation, and studies in explosive decompression.

THEORETICAL STUDIES

The Subcommittee on Decompression Sickness has taken the view that a purely empirical approach to the problem of decompression sickness will in the long run fall short and fail to meet the broader needs of military medicine, not only of aviation but of diving, salvage, and undersea construction. The problems of the diver are similar to those of the high-altitude flier, but a broad biologic approach to the problem indicates that there are some fundamental differences, notably that bubble size in divers coming up from great pressures is influenced almost entirely by nitrogen and little by carbon dioxide, whereas bubble size at altitude and the initiation of bubble formation may be greatly influenced by dissolved carbon dioxide. Thanks to the lively interest and co-operation of the United States Navy in our research program, it has become possible to co-ordinate and consolidate basic research on divers' bends and on altitude bends. These fundamental physiological studies fall into several categories: histology of bubble formation, with particular reference to primary site; conditions under which bubbles form in biologic systems; the physiology of inert gas exchange; and deformation pressure.

HISTOLOGY OF BUBBLE FORMATION

The work of Whitaker and Blinks in California reported in 1943 for cold-blooded animals and the similar work for mammals by E. Newton Harvey indicated that visible bubbles can be detected in veins carrying blood from muscles that have been thrown into active contraction. Search by Scott, Knisely, and others for the actual site of origin of these bubbles was disappointing; recently, however, through application of an ingenious freezing technic, Gersh and Catchpole at the Naval Medical Research Institute in Bethesda have shown that in high-pressure bends bubbles can be detected in the fat depots and also in the blood vessels coming from them. In altitude bends copious bubble formation has been demonstrated in the vessels coming

from muscle as well as from the fat depots, and it is their opinion that the blood vessels are a primary site of aeroembolism. Extravascular bubbles have also been described, and their presence around tendon sheaths and in joints has been established through the x-ray studies of Ivy and Ferris and more recently by work carried out at Berkeley. Some difference of opinion still exists as to whether bends symptoms or chokes are symptoms due in the first instance to intravascular bubble formation or to pain originating from extravascular bubbles.

Factors Underlying Bubble Formation in Biological Tissue

Harvey and his group have recently published six papers dealing with the theoretical aspect of bubble formation in tissues.[2] A comprehensive treatment is given of the various factors involved in separation of a gas phase from a liquid phase and the conditions for stability and growth of gas nuclei. Preoxygenation, anoxia, exercise, passive movement, and trauma, which may hinder or favor the formation of bubbles in the circulatory system of cats, were investigated in detail. Studies indicate that the gas tension of cats must be increased to between 2.6 and 3.1 atmospheres before spontaneous intravascular bubble formation occurs at 45,000 feet. This corresponds to a ΔP of 2.5 to 3.0 (ΔP = dissolved gas tension — hydrostatic pressure). Previous studies have established that a similar ΔP is necessary for spontaneous bubble formation in cats decompressed to 1 atmosphere from previous exposure to compressed air.

The work of Ferris, Behnke, and Harvey indicates that the rate of denitrogenation varies widely from tissue to tissue and also depends on the state of activity. In a given species the rate of denitrogenation of the body as a whole may be correlated with the animal's specific gravity — in other words, with the relative amounts of fat in relation to the rest of the tissues. Susceptibility to decompression sickness is also closely correlated with specific gravity and hence with adiposity.

Inert Gas Exchange

Lawrence found by saturating normal persons with xenon, krypton, and nitrogen and following the desaturation that all the rates of desaturation were the same. In other words, diffusion is apparently not a limiting factor in gas exchange. This is a surprising finding and needs verification, since the observations of Behnke and his associates, comparing helium and nitrogen, indicate that diffusion rate is an important factor in gas exchange. Similarly, Lawrence and his group have found that subjects and animals partially

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[2] These references are listed in the bibliography at the end of this volume.

saturated with krypton or xenon have experienced preanesthetic sensations. This is probably due to the greater solubility of these two gases in both the water and the fat of the body.

DEFORMATION PRESSURE AND DECOMPRESSION SICKNESS

Recently Nims has developed a physical theory of decompression sickness, which accounts for the quantitative data on this type of sickness. This theory is based on the fact that gaseous bubbles growing in tissues must displace and deform adjacent structures. Because of the so-called "quasi-elastic" properties of the tissues, this deformation leads to an additional pressure, conveniently designated as a deformation pressure. If the deformation pressure (D) exceeds a threshold value (D*), nerve fibers or endings are stimulated by the mechanical deformation of the tissues. The intensity of the stimulation is proportional to the excess of D above D*. Mathematical development of this assumption leads to equations that satisfactorily account for the effect of altitude on the incidence of decompression sickness, the rate of production, and the time course of incidence of decompression sickness at altitude, and the effect of varying periods of denitrogenation on the incidence of symptoms. In terms of this theory, decompression sickness appears to be largely due to extravascular bubbles growing in "tight" tissues. The more important physical variables concerned in decompression sickness appear to be the partial pressure of dissolved nitrogen in the tissues, the apparent diffusion constant of nitrogen from the tissues to the alveolar air, the apparent diffusion constant of nitrogen from the tissues to a growing bubble, and the so-called "volume-elastic" properties of the tissues. Decompression sickness is the result of the differential pressures developed around gaseous tissue bubbles, and does not differ in kind from the symptoms of extreme gaseous distention, the pains of the blocked sinus, or the pains produced by excessive stretching of the tympanic membrane of the ear.

PRACTICAL APPLICATIONS

In addition to the more purely academic approach to the problem of bubble formation in tissues, the Subcommittee on Decompression Sickness was asked by the Navy to study a number of immediately practical problems involved in the selection of personnel for high-altitude operation and also for diving and salvage procedures. The Navy was also interested in a second practical problem; namely, how persons who are susceptible to decompression sickness could be protected and also how they might be tided over an acute bends episode — for example, if a single member of a bombing crew were affected at a time when it was inexpedient to descend to a lower altitude. The general research program fostered by the Subcommittee actually

divided itself into three primary groups — problems of selection, protection and first aid, and a third general problem put to us by the Army Air Forces somewhat later in our deliberations — that of explosive decompression, both in relation to bends and in relation to the physiological effects of such an accident on flying personnel.

PRESELECTION

With research units situated in nine civilian centers — namely, California, Chicago (two groups), Columbus, Rochester (Minnesota), Cambridge, Philadelphia, New Haven, and New York — various aspects of the problem of selection were simultaneously studied in seven of these centers. The early experience with selection technics in Canada and at Pensacola and Randolph Fields had indicated that great variation existed in the same person at different times, and that many runs to altitudes of 38,000 feet or above would be required to establish susceptibility in given subjects with any degree of certainty. At that time many of the factors involved in the precipitation of bends — that is, age, exercise, temperature, and physical fitness — were little understood. The Subcommittee accordingly sought to discover why individual susceptibility varied, and it was soon disclosed that if factors such as temperature and exercise, and also the time of day, were kept constant, there was much less variability. If a group of young adults in good physical condition were subjected to vigorous exercise at an altitude of 38,000 feet, they all ultimately came down with bends, but the susceptibles came down promptly, whereas those who were more resistant succumbed only after a considerable interval. With conditions thus controlled and exercise introduced as a constant factor, it became possible to classify persons on a satisfactory and reproducible basis.

On the basis of this experience a selection procedure was developed by the Subcommittee on April 30, 1943, which involved only one ninety-minute exposure at altitude, but the Subcommittee offered as an alternative a more exacting test involving three such altitude exposures; the latter test was proposed for those assigned to special and more exacting duty (reconnaissance); the former and briefer test was used for general over-all classification of flying personnel. The details of the single ninety-minute test are given in the following protocol.

A Ninety-Minute Preselection Test for High-Altitude Operation [3]

The considered opinion of the Subcommittee is as follows:

(1) Oxygen indoctrination for flying personnel should be carried out separately from the high-altitude selection test, and the oxygen indoctrination run (or runs) should be conducted first.

[3] Recommendations drafted by the Subcommittee on Decompression Sickness on April 30, 1943, and endorsed by the Committee on Aviation Medicine on May 3, 1943.

(2) The preselection test should consist of a run to 38,000 feet at 5000 feet per minute, with oxygen from the ground up. When the altitude is reached, subjects should perform three deep-knee bends and then raise the hands over the head three times, lifting in each hand weights of 3.5 pounds (for example, oxygen cylinder caps), repeating this every ten minutes for ninety minutes, or until definite bends or chokes develop. Records must be kept of the time of onset of bends in all subjects and of the time of descent; subjects should be instructed to cease exercise as soon as the pain of bends begins or chokes develop.

(3) Classification by the test is as follows:

Group A — not incapacitated in 90 minutes; that is, very resistant.

Group B — descent in 60–90 minutes; that is, resistant.

Group C — descent in 30–60 minutes; that is, less resistant.

Group D — descent in 0–30 minutes; that is, susceptible.

It is anticipated that Groups A and B will be suitable for prolonged flights above 30,000 feet and will constitute between 30 and 50 per cent of subjects.

(4) For purposes of validation, the test just outlined should be repeated on 300 or more subjects two or more times. Time intervals will be subject to change on basis of validation tests.

(5) If this classification gives the necessary number of very resistant individuals, the Subcommittee intends to have tests carried out repeatedly on single individuals, further to appraise the validity of the test.

A number of modifications of the test were studied. The group directed by John Lawrence in California believed that more reliable results could be obtained if exercise consisted of stepping up on a 1-foot ledge a set number of times in the place of the deep-knee bends originally proposed by the Subcommittee. When the alternative forms of exercise were under discussion, the armed forces let it be known that the need for pilots was so great and the turnover during training so rapid that they had become more concerned over methods of protection against bends than they were in selection procedures. The information obtained in the selection studies was looked on as highly significant, but it was set aside to be used for personnel intended for special duty, such as the deeper diving operations and high reconnaissance flying, rather than for the rank and file of service flying personnel.

Radioactive Indices

Before dismissing selection procedures, a word must be said about another quite different approach to the problem, which was developed by Lawrence and his group at Berkeley. This involved study of the rate of transmission of certain radioactive inert gases through the lungs and into the tissues. It was found that persons who eliminated the inert gases rapidly were less susceptible to bends than those whose tissues tended to retain these gases and to eliminate them slowly. By studying the rate at which radio-argon is transmitted from the lungs into the tissues (as tested by a Geiger counter applied to one of the extremities during inhalation of the radioactive gases), an

index was obtained of the rate of inert gas transmission of the tissue of the given subject. When persons preselected in this manner were subsequently tested by the Subcommittee's ninety-minute chamber test, close correlation was found. But although the test was of great theoretical interest, it was beset by practical difficulties, since the radioactive argon isotope immediately available had a relatively short half-life, and unless the other isotope, which has a half-life of 38,000 years, could be made available it would be impracticable to use the test at any distance from a cyclotron. Since there were technical difficulties in obtaining the long-lived radio-argon, this test, like that of the ninety-minute chamber test, was pigeonholed against the time when it might be called for.

PROTECTION AND FIRST AID — APPLICATION OF PREOXYGENATION PROCEDURES

When it was recognized that altitude bends represented a clinical syndrome closely similar to divers' bends and therefore due principally to the formation of nitrogen bubbles in tissues and blood vessels, it became evident that breathing oxygen prior to flight, which would tend to eliminate nitrogen from the tissues, would be effective in protecting flying personnel from the hazards of decompression sickness. The practical question involved, however, was that of time. How long would the average man be obliged to breathe oxygen at sea level to be protected for a four-hour operation at 38,000 feet, or for one or two hours at this altitude? Similarly, if bombing operations were carried out at lower altitudes (for example, 30,000 feet), how much less time correspondingly must be spent in oxygen breathing to be ensured of adequate protection? To answer questions such as these involved studies of the rates of nitrogen elimination on large numbers of subjects, and it also involved knowledge of conditions of exercise, temperature, and so forth to which a given member of a bombing team or a fighter pilot would be exposed during maneuvers. It was soon established by Behnke and his collaborators that four hours of preoxygenation would completely protect any normal young adult from bends during four hours of vigorous work at altitude, but it was clearly impossible to subject bombing teams to this procedure, and it was absolutely impossible for fighters to breathe oxygen for four hours before ascent. Shorter time intervals were therefore studied, and it was found that an average group of healthy young adults, if they preoxygenated for thirty minutes and continued on oxygen during the ascent to altitude, would have a reasonable measure of protection for all altitudes and for nearly all intervals during which they would be exposed to bends altitude in an ordinary bombing operation.

As with preselection, however, it turned out as the war progressed that operations at extremes of altitudes were less common than had at first been

anticipated, so that preoxygenation was used more for special operations than for routine bombing missions. Men who proved bends-susceptible soon, by their own choice, arranged to preoxygenate before a mission; by process of elimination, therefore, the susceptibles, on the basis of information at hand, learned to protect themselves. Those who were not bothered by a given flight pattern ceased to preoxygenate.

The outcome of these studies has been gratifying from many points of view, because they gave needed information to those who required it, and a sense of security to those who might at any time be faced with the problem. In different theaters the flight patterns were different, and the incidence of decompression sickness therefore varied widely.

Validation of Chamber Tests and Preoxygenation Procedures

In the services there had always been a lurking doubt whether chamber experiments could be transferred to experiments in aircraft; that is, whether chamber tests for bends susceptibility would be applicable to personnel flying to corresponding altitude equivalents in a plane; similarly, there was some doubt as to whether preoxygenation procedures as tested in low-pressure chambers would prove applicable for personnel in aircraft. Through a co-operative undertaking between the Berkeley unit and the Research Division of Consolidated Aircraft in San Diego, a B–24 was made available for two months, during which Lawrence took his group of chamber-tested subjects to altitudes of 35,000 feet. During the flight they carried out exercises similar to those performed in the ninety-minute test. The correspondence in bends susceptibility in the chamber and in the plane was remarkably close, as indicated by the reaction of 47 subjects in twenty-five successive flights. The following conclusions were reached:

Of the "resistant" subjects (that is, those who had previously passed two chamber runs), all but one passed the plane flights, whereas in the "susceptible" group, all of them developed bends in flight and incapacitation usually resulted. The mean pain intensity (on a nine-point scale) of the susceptible group was 4.32, but that of the resistant group was 0.77. Chi-square and critical-ratio tests demonstrated that the difference in the performance of the two groups in the plane flights is very significant. Thus, chamber selection procedures do have a definite value in choosing an air crew to perform a given task at a certain altitude.

The Bends Bag

During these flights Lawrence was able, incidentally, to test the effectiveness of a pressure bag developed to deal with anyone developing an overwhelming attack of bends in the aircraft at a time when the experiment was still in progress. The men had several occasions to use the bag, and in each case it conferred immediate relief from bends symptoms. In one instance, however, a badly afflicted subject developed chokes and vomited while in the

bends bag, and this made it necessary to terminate the flight and to descend at high speed.

The validation of chamber experience by flight tests and proof of the effectiveness of the bends bag was also carried out independently by the Aeromedical Laboratory at Wright Field, with a B–17 as a test ship. Here again the Subcommittee has taken satisfaction in having established, with the co-operation of the aircraft companies and our associates at Wright Field, the validity of chamber testing, both as a preselection procedure and as a procedure for protecting personnel from the dangerous complications of decompression sickness. Seven independent civilian groups took part in these studies. They were all co-operative undertakings involving relatively large research teams under the following direction: John Lawrence of Berkeley; Walter Boothby of Rochester, Minnesota; A. C. Ivy, Northwestern University, Chicago; Eugene Ferris and M. A. Blankenhorn, University of Cincinnati; Detlev W. Bronk and M. G. Larrabee, University of Pennsylvania; Leslie F. Nims and John F. Fulton of Yale.

Treatment of Post-Flight Reactions

A number of severe post-flight reactions have been reported after chamber "flights," several of which have terminated fatally. These reactions can be divided into two categories: circulatory collapse or shock and focal or diffuse central neurologic reactions. Deaths have occurred only in the cases with circulatory collapse; however, both syndromes may develop in the same person. Post-flight circulatory collapse is most likely to occur in subjects who have previously had syncopal symptoms with decompression sickness at altitude. The severe post-flight shock can best be prevented by making certain that all subjects who have had syncopal reactions at altitude and those who are pale or unduly fatigued after descent are kept under observation until their blood-pressure response to sustained relaxed standing is normal. This may require overnight hospitalization. Severe post-flight collapse results in the typical clinical picture of shock — leucocytosis, hemoconcentration, and sustained hypotension. It should be treated as a case of shock with plasma, 100 per cent oxygen, warmth, and complete rest.

The post-flight neurologic manifestations are generally ushered in by the development of scintillating scotomas with varying degrees of amaurosis (visual field defects). This indicates involvement of the occipital lobe, due probably to vascular spasm. Such involvement may rarely spread throughout the cerebral cortex to produce paralysis and coma. Following these focal neurologic signs, headache, with or without nausea and vomiting, may develop. It has been shown that these neurologic reactions occur more frequently in subjects susceptible to migraine and that the reactions are similar to clinical migraine. Most of these reactions run a self-limited course and are not severe enough to require treatment. For the severe cases, there is evidence

that the initial stage in which focal neurologic signs are present should be treated with vasodilators, such as carbon dioxide and nitroglycerine. The secondary stage of headache, nausea, and vomiting if severe warrants the use of ergotamine.

Explosive Decompression

With the development of pressurized military and transport aircraft, but particularly with the development of the pressurized bomber and the jet-propelled rocket, and the hazards arising from sudden decompression such as might occur if a pressurized ship were suddenly rent by enemy fire or (as frequently happened in training operations) if a blister were blown out, it is important to know what effects such accidents will have on flying personnel. The problem was studied early in the war at Wright Field and the Subcommittee on Decompression Sickness sponsored a long-term contract on explosive decompression under the direction of Professor Fred Hitchcock of Ohio State University. He has worked in close collaboration with Major Sweeney, who was engaged on similar studies at Wright Field. Their studies indicate that explosive decompression at rates which might occur in larger pressure-cabin aircraft, does not significantly increase bends incidence, and such explosions as have been studied experimentally (and in accidents occurring in B-29's during training flights) do not seriously discommode flying personnel. The critical rates likely to cause injury are still under study, and the physiological changes resulting from the sudden decompression remain under active consideration. From combat operations we learn that B–29 flying personnel can carry out prolonged missions in their pressure cabin with far less fatigue than would be the case at corresponding altitudes breathing oxygen without pressurization. It is the considered opinion of all those in touch with B–29 operations that pressure cabins are here to stay, and that pressure will be used even when not flying at the extremes of altitudes, for this confers tactical advantage owing to the diminution of fatigue and the maintenance of greater alertness on the part of flying personnel.

In addition to giving an answer to the question set by the Air Forces, Dr. Hitchcock has obtained results of great theoretical interest and, in the course of his studies on explosive decompression, he has been able to secure alveolar air samples of greater purity than is ever possible with ordinary sampling in which the alveolar is mixed with the tidal air. The new data have lent new and welcome support to the view that the respiratory gases pass through the epithelium of the lungs by a simple process of diffusion governed by the particle pressure of the gases in question.

Part Four: Physiology

INTRODUCTION

JOSEPH T. WEARN

INVESTIGATION in the field of physiology was carried out by Division Four of the Committee on Medical Research. A wide range of subjects was covered. Under one set of contracts a broad study of the clinical, physiological, and chemical aspects of shock was carried out. Another study dealt with the chemical fractionation of blood and particularly of plasma, both human and bovine. A search was also made for effective blood substitutes, and when possible ones were found they were submitted to exhaustive study before clinical tests. The preservatives for whole blood and for red cells were carefully studied in vitro and in vivo. Finally, a number of investigations were carried out in a field that might be called environmental medicine. They dealt with the physiology of acclimatization, the effects of climate on the efficiency of troops and their nutritional and vitamin needs, and the corresponding effects of various altitudes. They also covered the physiological effects of clothing; that is, the effects of the various types of fabric on sweat loss and water and salt need, on the skin, and on efficiency in general. In this group of contracts also were those for studies of the sterilization of water in small containers. In some theaters of the war troops were obliged to get water where available and under fire, so that the need for a rapid, efficient sterilizing agent for water in small amounts was an urgent one.

In World War I, shock was one of the major factors that contributed to the high mortality rate from wounds. So important was the problem that outstanding physiologists from this country and from England were selected to study the subject, and the Surgeon General of the Army made it possible for some of these workers to carry on their studies at or near the front. In this war the Committee on Medical Research and especially its advisory committees planned extensive studies of the clinical, circulatory, and metabolic aspects of shock. The Committee's advisory groups, especially the Committee on Shock and the Subcommittee on Blood Substitutes, foresaw clearly the

need for whole blood and blood plasma for the prevention and treatment of shock, and the latter committee recommended repeatedly that plans be made for getting whole blood to the fighting lines in all theaters. Blood plasma was soon made available to every front. With its use the incidence of severe wound shock dropped to a low level, and when whole blood was finally made available in the Pacific and European theaters, the so-called "irreversible shock" ceased to be a problem of any import.

The vast, efficient program of the American Red Cross in collecting blood for processing into plasma made possible fundamental studies of the fractionation of human plasma. Bovine plasma was also studied. The work carried on in the laboratories of the Harvard Medical School, the University of Wisconsin, the California Institute of Technology, and Columbia University, with clinical testing units in New York, Philadelphia, Richmond, Atlanta, Boston, San Francisco, and various other places, demonstrated clearly the advantages of co-operative research. The studies of the constituents of blood plasma were among the most productive and significant of the medical researches carried on under contract with the Office of Scientific Research and Development.

The search for substitutes for whole blood and for blood plasma led to the trial of several substances and to the conclusion that there is no ideal substitute for either whole blood or plasma. It is a pleasure to point out the skilled, concise, and definitive work of the several teams of investigators who put to test and appraised the various blood substitutes.

After very careful studies, acid-citrate-dextrose solution (A.C.D.) was selected as the most effective preservative available for whole blood. This solution preserved whole blood for at least three weeks, and this made it possible to ship whole blood by air to the Pacific and European theaters. In a splendid co-operative research, several investigators in Boston, Cincinnati, and Bryn Mawr demonstrated conclusively in less than ten weeks' time that A.C.D. solution was an adequate preservative when the blood was properly refrigerated. The co-operation of Captain Lloyd Newhouser of the Navy Medical Corps was most helpful in the final testing of the preservative under field conditions. The need for blood preservative was acute. The rapid and thorough manner in which it was tested and delivered was one of the outstanding examples of effective co-operative investigation in the medical field during the war.

The contracts recommended to the Office of Scientific Research and Development by the Committee on Medical Research in the following subjects were the results largely of requests to the Committee from the offices of the Quartermaster General and the Surgeon General of the Army. The Chief of Division Four served as liaison officer and consultant to the Quartermaster General, especially to the Military Planning Division under Brigadier General Georges F. Doriet, and as consultant to the Surgeon General in the

Division of Preventive Medicine under Brigadier General James S. Simmons. These officers' keen appreciation of the help to be had from the proper use of research laboratories and their constant effort and forethought to improve the well-being and efficiency of the American soldier were an inspiration to the staff of this division.

Early in the war the Subsistence Branch of the Military Planning Division of the Office of the Quartermaster General requested the help of the Committee on Medical Research in determining the effect of dehydration, treatment with sulfite, storage at high and low temperatures in dry and moist climates, and processing and cooking on the palatability, vitamin content, and keeping qualities of various foods. These contracts were under the supervision of a special subcommittee of the National Research Council. The results of these studies furnished information of great value.

In the spring of 1945, the Chief of Division Four and Colonel John B. Youmans of the Division of Preventive Medicine were sent by the Surgeon General of the Army to survey the state of nutrition of American troops at various stations in the Pacific Theater. The survey was completed by a team made up of medical officers and a consultant to the Surgeon General equipped for carrying out the necessary tests to determine the state of vitamin nutrition. It was reassuring to find no vitamin deficiency in any of our troops.

It became obvious early in the war that American troops would have to serve under extremes of climate ranging from that of Alaska to those of New Guinea and the African desert. Hence, it was imperative to equip, train, and condition troops for such climates. Work was undertaken at the request of the Quartermaster General to ascertain the effects of extreme heat and cold, so that steps might be taken to counteract the ill effects and maintain the efficiency of troops under such conditions. Our investigators carried out their work at the University of Rochester, the Harvard Medical School, and the University of Michigan, as well as on the field.

For the first time in the history of warfare a careful and thorough investigation of the effects of hot- and cold-weather clothing on the physiology of man was carried out. Much was learned, and much remains to be learned. These studies were made at the request of the Office of the Quartermaster General. The physical properties of textiles were determined before making them into uniforms, which were then investigated in the laboratory and finally in field tests. Dr. Sid Robinson of the University of Indiana and the staff of the Harvard Fatigue Laboratory rendered valuable service, both in the laboratory and in the field.

The Quartermaster General, with the endorsement of the Surgeon General of the Army, requested the Committee on Medical Research to make a search for a simple single tablet for the sterilization of water in canteens and Lyster bags. The request was also made that a long-time study be undertaken

to determine the mechanism by which chlorine causes the death of bacteria. After a survey of previous work, the decision was reached to set up a single laboratory equipped with adequate personnel and facilities for the over-all testing of available water-sterilizing agents, and to explore the possibility of the use of new compounds. Professor Gordon M. Fair of Harvard University headed the laboratory, and Dr. David Green of New York undertook the study of the mechanism by which chlorine kills bacteria. In a relatively short time light was thrown on this mechanism and a new tablet for the sterilization of water was produced, which was adopted for official use by the United States Navy and Marine Corps.

SHOCK

I. The Clinical Aspects of Shock

DICKINSON W. RICHARDS, JR.

THE VARIOUS investigations of traumatic shock in man carried out during World War II under the direction of the Committee on Medical Research can be considered under four general headings: the mechanism of shock; the effects of certain therapeutic agents, particularly blood substitutes; special inquiries into vasomotor behavior and possible chemical or toxic factors; and the particular problem of thermal burns.

MECHANISM OF SHOCK

During the early months of the war, much emphasis was given to the search for reliable signs of early or impending shock. This search was not highly successful. Arterial blood pressure was confirmed as the best single measure of shock, although unreliable in many instances. It was agreed that the extent and duration of injury were the best guides to anti-shock treatment.

Increasing attention was given to the mechanism of shock, with the use of available physiological methods. One of the most important of these was the measurement of plasma and blood volume. During the latter part of World War I, loss of blood volume had been recognized as a factor of primary importance in shock due to injury. Improvement in method, through the use of the dye T–1824, revived interest in the subject in the years just before World War II.

Of all the findings in our clinical studies, one of the first to be demonstrated — the most striking discovery and undoubtedly the one of greatest practical significance — was that in shock following trauma the decrease in blood volume is regularly associated with hemodilution, that the loss from the circulation is that of whole blood, and that it occurs at the site of injury. This was reported as early as the spring of 1942. During the next two years it continued to be emphasized, along with the much greater weight of evidence coming in at the same time from the military theaters and the recommendations from the Subcommittee on Blood Substitutes of the National Research Council.

That the decrease in blood volume was due to the loss of whole blood at the site of injury, and not to exudation of plasma through a general increase in capillary permeability, appeared also from the work of two investigators, who showed that following skeletal trauma the quantitative losses of red cells and plasma protein from the circulating blood stream corresponded to a loss of whole blood only. Of significance also was the amount of blood loss in moderate and severe shock, the deficits being of the order of 1500 to 2000 cc. of whole blood, or 30 to 40 per cent of the circulating blood volume. This confirmed Keith's work in World War I.

Further information on the changes in the circulation occurring in shock was obtained by catheterization of the right side of the heart. This method, first used in Germany in 1929, was developed into a practical and safe procedure by Cournand and Ranges in 1941.

These technics, combined with other standard procedures, enabled comprehensive physiological studies to be carried out during the state of shock and during recovery from it. The measurements included plasma and whole-blood volumes; arterial, peripheral venous, and right-auricular pressure; peripheral resistance; arterial and mixed venous oxygen and carbon dioxide contents; pulmonary ventilation and oxygen consumption; cardiac output; arterial and venous pH, lactic acid, and other chemical measurements; renal clearances and renal blood flow; and extracellular tissue volumes.

In shock due to skeletal trauma or hemorrhage, the basic changes, in addition to loss of blood volume, were found to be decreases in right-auricular pressure, in cardiac output, and in arterial blood pressure. This was the physiological picture that had already been described in experimental shock in animals: loss of blood volume, leading to diminished venous return and diminished blood flow. In abdominal injuries and burns the findings were different in that instead of hemodilution there was hemoconcentration, more marked the more extensive and severe the injury; arterial blood pressure was also more likely to be maintained at normal levels in these conditions. Head injuries usually gave a different circulatory response, the arterial blood pressure being normal or increased and the blood flow accelerated.

Acidosis in severe shock was uncompensated and often marked, with the arterial serum pH between 7.2 and 7.1. This was, however, definitely a secondary phenomenon. It was not present immediately after injury but developed progressively, in untreated subjects, usually after the second or third hour. It was apparently associated with continued tissue anoxia. Blood lactic acid levels increased parallel with the decrease in pH.

In recovery from acute shock following adequate fluid replacement, the circulation was promptly restored in all except the severest and most prolonged cases of shock. With restoration of blood volume, the auricular and arterial blood pressure and the cardiac output increased and acidosis dis-

appeared. It was emphasized that for sustained recovery the transfusion of large amounts — a liter or more — of blood or plasma was required.

EFFECTS OF THERAPEUTIC AGENTS

Early in the course of these investigations comparative measurements were made of the relative therapeutic effectiveness of intravenous saline solution, plasma, and whole blood. It was found that saline in moderate amounts increased cardiac output but produced only a slight or temporary increase in blood volume, and that plasma or plasma substitutes brought a sustained increase in blood volume and cardiac output, but that there remained, as one would expect, a deficit of red cells. Total transport of oxygen to the tissues was thus still deficient, and tissue anoxia persisted. Only whole-blood transfusions restored oxygen transport and completely relieved tissue anoxia.

Throughout the war considerable effort was devoted to clinical studies of various blood and plasma substitutes that had been developed under the direction of the Subcommittee on Blood Substitutes and to comparison of these with standard agents — saline solution, plasma, and whole blood. Outstanding among these products were the concentrated human albumin solutions and gelatin solutions.

Concentrated albumin was found to be a practicable and effective plasma substitute. It was easily handled and could be introduced very rapidly. A combined study by four research teams demonstrated that when given without additional fluid the albumin retained fluid in the blood to the amount of 12 to 14 cc. per gram of albumin. When given according to clinical recommendation — that is, 25 gm. of albumin in 1 l. of saline — there was a further blood-volume increase, up to 17 cc. of fluid per gram of albumin. The albumin was found to remain in the circulating blood for several hours.

Lightly degraded gelatin of high molecular weight was also found to be an effective plasma substitute. One investigator considered it especially useful in the early treatment of burns.

VASOMOTOR BEHAVIOR AND TOXIC AGENTS

Even with the acceptance of traumatic shock as a critically diminished blood flow due to loss of blood volume, leading to progressive tissue anoxia, there remained many variations in the pattern of shock that could be explained only by assuming different states or different reactions of the vasomotor system. A fundamental relation here was the so-called "peripheral resistance," which is essentially the rate of fall in pressure along the vascular bed per unit of blood flow. This rate is expressed as mean arterial blood pressure divided by cardiac output per minute.

From this point of view a number of investigations were carried out, some to identify local or regional states of vasoconstriction or vasodilatation in shock due to different causes, others to analyze the over-all vasomotor status of various forms of shock, and still others to inquire into the possibility of toxic factors in shock, due to anoxia, tissue destruction, or infection, as effective in maintaining, if not causing, peripheral circulatory failure.

A special study of some interest in this connection was that of the renal circulation in clinical shock. It was found that the decrease in renal blood flow was proportionally greater in shock than the decrease in total blood flow, and that there therefore existed a marked degree of renal vasoconstriction. A secondary decrease in glomerular filtration led in severe cases to total anuria. These findings appeared to be related to the anuria of the crush syndrome.

From the point of view of total blood flow in acute oligemic shock, the renal vasoconstriction could also be thought of as a temporary compensatory mechanism, the blood normally flowing through the renal arteries being made available to more immediately vital regions such as the brain and heart. In this type of shock due to trauma or hemorrhage, it became apparent that there existed a compensatory vasoconstriction, the circulation through the extremities, for example, and through the kidneys, as already described, being greatly diminished for the benefit of the blood flow through more essential regions. With maintenance of this selective vasoconstriction, the circulation, although impaired, could continue; loss of vasomotor tone could result in abrupt and sometimes fatal collapse.

The influence of bodily position in shock was examined quantitatively, and the benefit of the well-known "shock" or head-down position was demonstrated, the chief effect being an improved arterial blood pressure. Alcohol was found to produce an unfavorable vasodilatation, with a fall in arterial blood pressure; sympathomimetic drugs caused a transient rise in arterial and venous pressure but with a decrease in cardiac output, with no clinical improvement; adrenocortical extract in large doses was without effect. The familiar unfavorable effects of long-continued shock, of exposure, and of physical movement or manipulation were confirmed.

Different forms of injury produced varying patterns of circulatory failure and shock. Chest injuries involving damage to the lung were usually associated with unsaturation of arterial oxygen, relieved by oxygen inhalation, contrasting with the normal saturation in most forms of shock without lung injury. Furthermore, too vigorous fluid replacement in cases of chest injury sometimes caused increased arterial anoxia, with or without pulmonary edema, suggesting acute pulmonary congestion.

A number of patients with chest injuries were studied in whom the state of hypotensive peripheral failure was greater than could be accounted for by blood loss or decrease in cardiac output. Arteriolar dilatation appeared to

be an important factor. Stab wounds of the heart, with cardiac tamponade, were also investigated. Tamponade reduced circulation by preventing venous return. Temporary improvement was effected in some of these cases by giving intravenous fluids, thereby increasing venous return to the heart even though this further elevated an already high venous pressure. Some cases were also relieved by simple pericardial aspiration.

In abdominal injuries, as already indicated, the usual finding was hemoconcentration, sometimes marked, due to passage of fluid into the peritoneal cavity, with the cardiac output decreased and the arterial blood pressure often near normal. The increased peripheral resistance that maintained arterial pressure was probably due partly to vasoconstriction and partly to the increased viscosity of the concentrated blood.

An investigation of the effects of phlebotomy on the circulation brought out two significant facts. First, a phlebotomy of moderate size (300 to 900 cc.) caused a fall in right-atrial pressure but no change in cardiac output, suggesting that the great veins hold a reserve of blood and that in normal subjects the right-atrial pressure is greater than is necessary for maintenance of cardiac output. Second, subjects who fainted in the course of phlebotomy suffered a marked fall in arterial pressure with no change in cardiac output and an actual increase in right-atrial pressure, indicating that the immediate cause of syncope is an acute loss of vasomotor tone, or more specifically an arteriolar dilatation.

During the last two years of the war, attention was again directed to the possible effects of toxic factors in the cause or maintenance of peripheral circulatory failure. This was brought about by several influences: the extension of interest to include the later stages of tissue injury, and circulatory damage, developing even several days after trauma; the renewed emphasis on the importance of vasomotor factors; and some of the advances in experimental studies of the effects of anoxia, tissue damage, and infection. The clinical investigation that was conducted in this field did not go far enough to provide any conclusive proof of the importance or even the existence of specific toxic factors.

Irreversible states in traumatic shock were also encountered, both in early hours after injury, when the latter was overwhelmingly severe, and on subsequent days, after initial resuscitation had been successful. In these cases restoration of the blood volume to normal did not restore and maintain the circulation. Detailed studies were not, however, made on a sufficient number of such cases to provide a definitive description.

It was at this point — the investigation of massive and complex injuries — that the shock program broke down. As indicated above, a technic had been developed for comprehensive study of the mechanism of shock and the results of treatment. On the other hand, the severest and most multiple forms of injury were being encountered only in the military theaters. The obvious

solution was to have research teams, using the newer technics, study military casualties. When permission to do this was requested from the Surgeon General's Office, however, it was refused. Thus it came about that no adequate investigations were made of many of the most serious and most important types of military injury — for example, severe chest wounds, extensive and multiple abdominal injuries, and thoracoabdominal wounds. Little or nothing was learned about the quantitative effects on the circulation of blood replacement in such cases or about blood flow, central and peripheral venous pressure, state of arterial and venous anoxia, or even pulmonary ventilation. When pulmonary edema developed, it was not known whether this was pulmonary or cardiac in origin. As little was learned about the cases that failed on the second or third day or later. The whole question of irreversible shock in man, which some of the severest cases probably represented, went by default. So far as exact knowledge of what happens to the circulation in such cases is concerned, this vast amount of clinical material was lost.

These statements do not detract in any way from the immense advances achieved in the treatment of severely wounded casualties. But they may serve to emphasize the wide gaps that still exist in knowledge of the shock picture in its most advanced state and in logical and fully adequate treatment.

THERMAL BURNS

Most difficult of all problems in the civilian studies was that of thermal burns. This was worked on extensively by groups in the Division of Physiology of the Committee on Medical Research and by others in the Division of Surgery. The problem extended far beyond shock as such and included the course of the patient over many weeks. In general, the acute oligemic state brought about by loss of plasma into injured regions was associated with marked hemoconcentration and markedly decreased cardiac output, but usually with sustained arterial and right-auricular pressure, indicative of vasoconstriction. Systemic treatment of burns at first consisted largely in administration of plasma. Subsequently, it was shown that twenty-four to thirty-six hours of fluid loss into the burned area, including both the burn itself and the surrounding edema, caused a salt (sodium)-and-water deficit in the body of several liters, best replaced by large amounts of intravenous and oral saline solution, up to 8 or 10 l. during the first forty-eight hours. It was interesting that the extracellular "sodium space" during the first thirty-six hours, as measured by radioactive sodium, was increased more than the "thiocyanate space," suggesting that some sodium in the burned regions was lost into injured intracellular tissues. A normal urinary output proved to be a useful index of adequate therapy. After forty-eight hours, while edema was subsiding, it was found that plasma volume was often increased and that

circulatory congestion might occur at this time if fluids were forced too vigorously.

The progressive anemia of severe burns, probably due at least in part to infection, required many transfusions, begun early and repeated, often over several weeks. Other measures, in addition to the difficult problems of local surgical care, included careful attention to food intake, maintenance of urinary output, and sometimes oxygen therapy in severe cases. In spite of the best efforts, the mortality remained high in cases with third-degree burns of more than 30 per cent of the body surface. The circulatory state in a patient failing from an extensive, heavily infected burn was one of tachycardia, slight hypotension, hypoproteinemia, anemia, normal or increased blood volume, and a tendency to edema, both systemic and pulmonary. At this stage neither transfusions, infusions, stimulants, digitalis, oxygen, nor other measures were usually effective.

SUMMARY

To conclude, one may state that the clinical studies of shock described above demonstrated the immediate mechanism of various types of traumatic shock, embraced quantitative measurement of the effects of different forms of therapy, and included an especially careful series of inquiries into the physical and metabolic effects of thermal burns. Many problems connected with the treatment of burns were left unsolved, and little progress was achieved in the study of the most massive forms of trauma and of irreversible types of shock.

II. Experimental Traumatic Shock

JACOB FINE

Well over twenty research projects were devoted to an experimental inquiry into the problem of traumatic shock. Although the title of each project embraced only a specific and limited phase of the problem, no restraint was imposed on the investigators to prevent their going as far afield as their findings or their speculations on the general subject indicated. The freedom of inquiry resulted in a flexibility of approach that proved fruitful in many directions.

The various phases of shock studied intensively were as follows: the hemodynamics of shock, including the question of capillary permeability; the relation of infection to shock; the role of the nervous system; the significance of the renal system, including a study of the renal injury in the muscle-crush syndrome; the effect of anesthetics and analgesics and of environmental tem-

perature; the intermediary metabolism; and the general and specific problems of therapy. Most types of shock were investigated, particularly hemorrhagic shock, shock resulting from muscle damage or tourniquets, and shock due to burns. A brief running account will be given of the more significant results achieved by these experimental studies, except clinical studies and those concerned with intermediary metabolism, which are discussed in the other sections of this chapter.

The primary aim of the collective study was to improve the therapy of traumatic shock in men wounded in battle. The great majority of patients in shock are in this state owing to the loss of whole blood. The therapeutic problem in most cases was therefore relatively simple: to provide whole blood quickly and in adequate volume. The probability that whole blood would not always be available required prodigious effort to develop satisfactory substitutes for it and sufficient quantities of plasma where plasma was preferable. The therapeutic products developed were of considerable value to those engaged in the experimental problem, which was basically to discover the best treatment of traumatic shock that fails to respond to correct blood-volume therapy. Its solution obviously demanded a more searching inquiry into the nature of the fundamental disturbance involved.

CAPILLARY PERMEABILITY

Between the first and second world wars much had been learned, chiefly about the hemodynamics of shock, but certain doctrines had gained popularity more by constant reiteration than by incontrovertible evidence. Thus, the frequent failure of blood-volume therapy led to the theory that tissue anoxia damaged the integrity of the capillary wall, and that the infused fluids did not remain in the circulation and could be found in the tissue spaces. Although the supportive evidence was of dubious quality, the theory was widely accepted. If it was correct, the therapeutic approach demanded by it must concern itself with healing the damaged capillary wall.

This discouraging prospect stimulated a new approach to the question of capillary leakage. The circulating plasma proteins were labeled with radioactive elements or heavy hydrogen; these substances were incorporated directly into the protein molecule, or plasma-deficient dogs were fed with amino acids containing them. Such proteins, injected into the blood stream of dogs in various types of traumatic shock, were traced and found to escape from the circulation at a rate not exceeding that in the normal dog. Further, the extravascular concentration of the plasma proteins in various tissues in shock was determined and found not to exceed that in the normal dog. Only in the terminal phase of advanced shock, when all tissues may be considered to have lost their integrity, was it clear that saline solution (and possibly, but not certainly, plasma) leaves the circulation more rapidly than it

returns. The evidence provided by two separate research projects, using independent although analogous methods, was strikingly in agreement that capillary permeability is not altered in traumatic shock, and that the reason for the failure of the organism to respond to blood-volume therapy must be found elsewhere.

HEMODYNAMICS

Since gross distortion of the hemodynamics is the most overt expression of the shock syndrome, it was natural that this field of inquiry should receive exhaustive attention before extensive search for the cause of therapeutic failure was made outside the circulation. Accordingly, the blood volume was studied intensively. Since infused fluids do not leave the circulation faster than in the normal state, they should be accounted for within the circulation by blood-volume measurements. The technic of the dye-plasma-hematocrit method depends on dilution of the dye by the total blood mass. Discrepancies between estimated and determined whole-blood and plasma volumes in advanced shock were at length discovered to be due to inadequate mixing of the dye with the total blood mass within the time limits that suffice for this purpose in a normal circulation. It appeared that false dilution curves were due to the sluggish state of the peripheral circulatory bed. The evidence of this state, although considerable, was amplified further as follows.

The accuracy of the dye-plasma-hematocrit method for determining total blood volume depends on its utilization of the hematocrit of the blood of large vessels for measuring red-cell volume. Gibson et al. presented evidence "to prove beyond question that the blood volume calculated from the determined plasma volume and the hematocrit of blood samples drawn from auricle, arteries, or veins is always higher than the true blood volume in the normal state. This is because the hematocrit of that portion of the blood circulating through the minute vessels is about half that of the blood in large vessels. The hematocrit of all the blood in the body is always lower than the large-vessel hematocrit. Thus, the dye-plasma-hematocrit method gives values that are from 10 to 30 per cent too high." The error is even greater in advanced shock because of "the greater spread between auricular and minute-vessel hematocrit. . . . Regardless of hemoconcentration or hemodilution, the magnitude of this spread is such that the error . . . is consistently from 20 to 40 per cent too high," so that significant changes in red-cell volume cannot be detected.

A new method of measuring red-cell volume was needed. This was provided by substituting for the dilution of dye that of infused red cells labeled with radioactive iron. By utilizing this method together with other technics, the reduction in circulating red-cell and plasma volume in excess of a known

loss was accounted for within the swamplike peripheral vascular bed.

This swamplike state of affairs in the peripheral circulation might be inferred from a variety of data: (a) the steadily declining venous oxygen concentration; (b) the increasing divergence in arteriovenous plasma pH values in favor of an increasing acidity on the venous side of the circulation; (c) the increasing divergence in the arteriovenous plasma carbon dioxide values due to the lesser fall in carbon dioxide concentration on the venous side of the circulation; (d) the obviously slower and even absent flow through much of the peripheral bed in deep shock, as seen by capillary microscopy in the dog's omentum and the rat's mesentery; and (e) the reversal of all these changes in the direction of normal during the transient recovery phase following a large infusion of saline or plasma or blood.

Quantitative proof of the fact that blood in the peripheral vessels was stagnant or trapped was demonstrated as follows. Morphinized dogs were given an intravenous dose of red cells containing radioactive iron (47-day half-life), and the red-cell volume was determined by the same progressive-dilution technic as is used for ascertaining plasma volume by the dye method. The plasma volume was measured at the same time. Bleeding sufficient to produce shock was then performed, and after the animal had been in shock for some hours an additional dose of radioactive red cells was given. In a number of experiments the resulting increase in radioactivity per cubic centimeter of cells was more than the expected amount. The red-cell volume calculated on the basis of these data was less than the expected one, allowance being made for the volume of red cells removed by bleeding. This is regarded as evidence of stagnation of red cells somewhere in the peripheral bed.

Further evidence of stagnation or trapping was obtained as follows. Dogs in hemorrhagic shock received a dose of radioactive red cells. On death the number of red cells in cubic centimeters per gram of tissue was determined by two methods:

(A) $\dfrac{\text{Hb. in 1 gm. of tissue}}{\text{Hb. in 1 cc. of arterial blood (just before death)}} \times$ arterial hematocrit.

(B) $\dfrac{\text{Radioactivity (Fe*) per gram of tissue}}{\text{Radioactivity (Fe*) of 1 cc. of arterial blood}} \times$ arterial hematocrit.
 (just before death)

Formula A is a method of direct determination of the total number of red cells in cubic centimeters per gram of tissue, but formula B is a determination of the true number of red cells in cubic centimeters per gram of tissue in circulation sufficiently active to have reached the tissues from the time of injection until death. The discrepancy between the results of the two formulas is a measure of the incompleteness of the mixing and therefore of the

extent of trapping of blood. The average ratio of formula B to formula A was found to be 1.0 ± 0.1 in 9 normal morphinized dogs. The average ratio in the shocked dogs was 0.7 to 0.8, indicating that some 20 to 30 per cent of the red-cell volume in tissues was trapped in the peripheral bed; that is, bypassed by the active stream.

Such a degree of trapping could not be demonstrated with equal uniformity for plasma. Possibly this is due to a partial balancing of trapped plasma by mobilized extravascular fluid. It may also be that in shock the mixing of plasma in active circulation with that in the peripheral bed is physically easier than is the case for particulate material such as red cells.

As shock deepens the trapping may be expected to increase. In later experiments the technic adopted for measuring the volume of trapped blood was altered in order to avoid errors inherent in hemoglobin measurements for determination of blood content. This was done as follows. A dose of radioactive red cells, containing radioactive iron with a 5-year half-life, was injected during the pre-shock phase, and when the dog was in late shock a dose of radioactive red cells with a 47-day half-life was injected. The latter, insofar as it might fail to mix completely, would show a deficiency as compared to the previously determined total red-cell volume, due to trapping.

Analysis of the tissues for the two types of radioactive iron was then made, and the number of red cells in cubic centimeters per gram of tissue was calculated on the basis of the content of each type of radioactive iron. The two determinations could be expected to agree if complete mixing occurred when both types of radioactive red cells were injected. If stagnation was present when the second dose (47-day half-life radioactive iron) was injected, the number of red cells in centimeters per gram of tissue, calculated on the basis of 47-day half-life radioactive iron, would be less in proportion to the degree of stagnation. This was found to be the case.

On an average, the tissue content of 47-day half-life iron was some four fifths of that of 5-year half-life iron. Dilution of the second dose of radioactive red cells was likewise approximately four fifths of the expected amount. The ratios by both technics indicated that there was stagnation of some 20 to 30 per cent of the red-cell volume in tissues.

The significance of these findings is that some 25 per cent of the tissue cells are suffering an oxygen deficiency and that functional collapse is inevitable unless relieved within a few hours.

Stagnation in the peripheral vascular bed is a generalized phenomenon and is not confined to damaged tissues, since it occurs in shock due to simple bloodletting and in infectious shock in which there is no localizing process, and is visually observed in tissues distant from the site of injury in traumatic (muscle-crush) or burn shock. Thus it is clear that the essential deficiency in shock that does not respond to blood-volume therapy is not inadequate blood volume but inefficient circulatory distribution of the total

blood volume, such that even when the latter is more than adequate, the volume and velocity of flow through capillaries is grossly deficient.[1]

The nature of the disturbance in the peripheral circulation is graphically depicted by Chambers and his collaborators, who made continuous microscopic observations of the exteriorized living omentum, which showed that the deficiency is progressive and therefore inevitably fatal. They describe the sequence of events as shock progresses as follows. Following an initial large hemorrhage, equivalent to 3.5 per cent of the body weight, the arteries and veins contract, the frequency and amplitude of vasomotion (rhythmic contraction and dilatation) in the arterioles and precapillaries increases, and the normal sensitivity to epinephrine is increased. After a prolonged period of hypotension, maintained for some hours by graded hemorrhage, a pronounced slowing of flow occurs, the venules dilate, vasoconstriction lessens, and sensitivity to epinephrine declines. During the vasoconstrictor phase, the terminal capillaries are relatively ischemic and more blood is shunted through direct arteriovenous communicating channels in order to sustain venous return. Eventually the arterioles and precapillaries lose tone, with resulting sluggish flow and finally stagnation of red cells in the capillaries and in the dilated atonic venules. The much reduced active flow continues to bypass the capillaries through direct arteriovenous channels. Many capillaries are therefore virtually out of circulation. The larger vessels remain constricted until death. The final phase of pooling or trapping of blood in capillaries results from loss of muscle tone in arterioles and precapillaries and loss of sufficient pressure to sweep the blood forward into the veins.

This process is induced by a severe depletion of circulating blood volume. It is essentially the same whether achieved by simple blood loss or by plasma loss following the release of tourniquets applied for many hours. There is no reason to believe that the situation is substantially different in any other types of traumatic shock. Resumption of normal flow is achieved by correcting the volume deficiency within a certain period of time; failure of response occurs if the infusion is given too late. In tourniquet shock delay is tolerated much less than in hemorrhagic shock, for reasons to be given below.

Irreversible shock, then, is a function of time. In other conditions — for example, shock due to overwhelming infection — irreversibility is not a function of time, since no large loss of circulating volume may be present and a favorable response to transfusion, no matter how soon it is given, does not occur and indeed is not to be expected. Thus the paralysis of peripheral circulatory function may be produced by more than one agent. Whether

[1] It has been stated that "reduction in the volume of blood returned to the heart is the keystone of all modern conceptions of shock." The recent evidence indicates that reduction in volume return is secondary to progressive pooling in the peripheral circulatory bed. The latter also explains the decreased bleeding volume in shock, which is, so to speak, another expression of reduced volume return to the heart.

such agents act directly or indirectly on the peripheral vascular mechanism has been the subject of considerable study and will be referred to again below.

THE NERVOUS SYSTEM

Among the possible mechanisms involved in the damage to the peripheral vessels, the role of the nervous system has been extensively studied, with little evidence to incriminate it.

Phemister produced neurogenic shock in experimental animals by prolonged stimulation of the cardioaortic nerves. This caused marked hypotension, reduced blood oxygen and carbon dioxide concentration, and exhaustion of the vasomotor center after four to seven hours, but removal of the stimulus at any time before collapse of the vasomotor center was followed by rapid recovery. These effects were not elicited by prolonged stimulation of somatic nerves. Nothing paralleling these experimental conditions occurs in man. The neurogenic factor is of little or no significance, except that if hemorrhage sufficient to lower the pressure to shock levels is followed by central vasodepression induced by direct nerve stimulation or abdominal manipulation, shock may be intensified. But prolonged hypotension alone in the absence of blood loss is tolerated very well.

Swingle revived the theory that nociceptive stimuli from the area of injury induce or intensify shock, on the ground that spinal anesthesia markedly reduces the incidence of shock resulting from muscle trauma to an extremity. Local procaine block provides protection to a lesser degree. However, section of the spinal cord or the main nerve trunks to the extremity does not prevent shock. The extent of local fluid loss was not measured and the hypotensive effect of spinal anesthesia was not considered. Phemister later demonstrated that the protective effect of spinal anesthesia is due to decreased bleeding into the injured area consequent on the reduction in blood pressure, and that the same protection can be provided by preliminary ligation of the large artery supplying the injured area in the absence of spinal anesthesia. Prior section of all dorsal spinal nerve roots below the twelfth thoracic provided no protection.

TOXIC FACTORS

That severe infection can precipitate shock is a common clinical observation. The induction of all the features of traumatic shock by bacterial toxins has been amply demonstrated in the experimental animal; the predisposition to shock in patients with infection subjected to surgery is well known. Recently Morton and Mahoney have shown that dogs suffering from a localized infection go into shock in response to a lesser degree of trauma or blood

loss than do normal dogs. Presumably infection, like malnutrition, cachexia, and so forth, deprives the organism of some as yet unknown principle whose function it is to resist the onset of peripheral circulatory collapse.[2] The bacterial toxins of *Clostridium welchii* and related organisms were found in the fluid collected from a muscle in the dog, following the release of its occluded blood supply (Aub et al.). Such toxins can induce typical traumatic shock. If they are given intravenously no substantial fall in plasma volume results; if they are given intramuscularly plasma loss and hemoconcentration occur. The shock-inducing effect of such toxins is not so severe if blood loss is avoided, thus serving to emphasize the additive effect of one etiologic agent on another. In an effort to localize the site of action of the Clostridia toxins, it appears that the toxin acts by splitting lecithin (in red cells and elsewhere).

In a heart-lung preparation, the declining cardiac output following the administration of *Cl. welchii* toxin was found to be due to damage to pulmonary capillaries and not to cardiac damage, for cardiac reserve was well sustained, as indicated by improvement in cardiac output, even in late shock, in response to transfusion. The latter observation has been made repeatedly in all types and degrees of shock until the prelethal phase. Thus, it is generally agreed that failure of cardiac function is not of primary importance in shock, in spite of the progressive decline in cardiac output. The evidence adduced by Page and especially by Wiggers that a measure of myocardial weakness exists in shock may be regarded as an index of damage from prolonged anoxia, which must affect the function of all tissues adversely. No toxin capable of producing shock has been shown to do so as a result of a direct effect on the heart, with the possible exception of the diphtheria toxin. (These observations do not of course exclude the possibility of shock developing as a result of cardiac damage. Shock following coronary thrombosis, myocardial infarction, or cardiac tamponade is a well-recognized clinical syndrome.)

Many investigators believe that nonbacterial toxins of endogenous origin are capable of producing traumatic shock. Kallikrein of tissue origin can produce shock, but there is no evidence that it is set free in the circulation during shock. An endotoxin from damaged muscle has been postulated. Page believes that the renal damage from muscle-crush injury is due to hemoglobin derivatives or to myoglobin from damaged muscle, but no convincing evidence that renal injury is related to the shock that may develop from the muscle trauma has been brought forth. Prinzmetal regards the phenomenon

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[2] The concept of resistance to shock and the development of immunity to the shock-producing effects of trauma has been demonstrated in rats, which survive prior exposure to a sublethal number of falls in the Noble-Collip drum. This immunity can be enhanced by prior forced feeding with a high-protein diet. It appears that sublethal trauma sets up a reserve supply or permits a rapid mobilization of metabolic reserves that counteract the energy-depleting effects of trauma.

of trapping in the peripheral circulation as due to a toxin, because congestion of the kidney and heart muscle and decreased bleeding volume occur in the rat within one minute after producing a hot-water burn. Chambers and his collaborators believe that the hyporeactive state of the arterioles and precapillaries in late shock is due to a circulating vasodepressor agent, since the blood of shocked dogs reproduces the same state transitorily in the mesenteric circulation of the rat. Shorr et al. have demonstrated a vasodepressor principle in ice-cold saline washings of liver from shocked animals. This principle is not demonstrable in other tissues except in muscle in lesser concentration and in blood in still smaller concentration. These investigators isolated the vasodepressor substance not only from liver tissue from shocked animals but also from normal liver kept in an oxygen-free environment for more than two hours. This substance, moreover, was inactivated by normal liver tissue and by anoxic liver after re-exposure to oxygen for two hours. This led them to regard the peripheral vascular collapse as due to the action of a vasodepressor substance derived from anoxic liver.

That the liver is seriously and progressively damaged in shock was suggested by the demonstration of decreasing ability of liver tissue of the rat to resume normal oxidation function in vitro in proportion to the duration of application of a ligature on the hepatic artery. In shocked dogs the excretion of bromsulfonphthalein by the liver is deficient. Further evidence that damage to the liver is related to the peripheral vascular collapse will be given below.

Such a postulate is more logical than the theory of a nonbacterial toxin derived from damaged tissue, since the peripheral collapse is present in a variety of shock states in which no overt tissue damage exists, except as the prolonged anoxic state eventually damages all tissues. Nevertheless, it has been argued that the more precarious state of a dog in tourniquet shock (in which muscle is severely damaged) as compared to dogs in hemorrhagic shock is due to the absorption of toxins from the site of injury. This conclusion seemed to follow from the observation that adequate volume-replacement therapy was ineffective in dogs in shock following release of tourniquets applied for five hours. Swingle showed that the application of plaster casts immediately after release of the tourniquet to prevent swelling prevented shock. Since the circulation was not interfered with, toxins, if present, should have been absorbed. It was later shown that if barbiturates are omitted shock is mild or absent when tourniquets are released after application for five hours, and that the failure of volume-replacement therapy is due to the intensification of the shock state by the barbiturates used as the anesthetic agent. When tourniquets were applied for eight to eleven hours without barbiturates (morphine only being used during the early phase of tourniquet application), deep shock resulted. But even this degree of shock responded favorably to volume-replacement therapy. Since the thera-

peutic agents used — plasma, 5 per cent bovine albumin, and so forth —
are incapable of neutralizing toxic agents, the theory of shock due to a
locally elaborated toxin received no support. It was thus demonstrated that
plasma loss constitutes the major if not the exclusive etiologic factor in
tourniquet shock.

BLOOD VISCOSITY

The important differences between tourniquet shock and hemorrhagic
shock, which have suggested a different etiology in the two conditions, are
more satisfactorily explained on a hemodynamic basis. These differences are
as follows:

(1) Transfusion is curative in hemorrhagic shock even after the blood
pressure remains at 30 mm. of mercury for several hours; it is ineffective in
tourniquet shock if the blood pressure falls below 80 for a similar or shorter
period.

(2) The sensorium is not dulled in hemorrhagic shock until the blood
pressure falls well below 40; it is dulled in tourniquet shock at pressures
below 100.

(3) Blood sampling is tolerated very well in hemorrhagic shock until
extremely low pressures are reached, but is poorly tolerated in tourniquet
shock at pressures between 80 and 100.

The blood deficiency is that of whole blood in hemorrhagic shock and
that of plasma in tourniquet shock. As expected, therefore, the hematocrit
is from 65 to 85 per cent in tourniquet shock, although normal in hemor-
rhagic shock. This is the most striking difference between the two con-
ditions. Since successful therapy in tourniquet shock is usually accompanied
by restoration of the high hematocrit to a normal or less than normal value,
it seemed likely that the more precarious state of tourniquet shock could
be explained by the added burden of increased viscosity on the vascular dy-
namics. Experimental inquiry showed the following:

(1) When the cardiac output is lowered by a fall in blood volume, an
increase in viscosity lowers it still further.

(2) The poorer tolerance to bleeding in tourniquet shock is due to the
fact that with increased viscosity the cardiac output is lowered to the critical
level — that is, one just barely compatible with survival — after a smaller
blood-volume loss than is the case in hemorrhagic shock.

(3) In the presence of a reduced blood volume, the reduction of a high
viscosity to normal or less than normal value improves cardiac output, but
not enough to improve the shock state noticeably. Volume deficiency is by
far the greatest danger, and its restoration is a more pressing need than is
that of a normal viscosity. If an abnormally high viscosity exists in shock and

is not treated effectively, the need for correction of a deficient blood volume is all the more urgent.

(4) At any level of reduced cardiac output the arterial blood pressure is higher when the viscosity is elevated, so that a deceptively favorable impression of the state of the circulation is given by the blood-pressure reading. At blood-pressure levels consistent with only a mild degree of hemorrhagic shock, the cardiac output in tourniquet shock is already disproportionately low. Hence, the increased hematocrit of tourniquet shock is a liability over and above that of volume deficiency and accounts for the major differences between tourniquet and hemorrhagic shock. The development of irreversible shock at higher levels of blood pressure in tourniquet than in hemorrhagic shock is explained by these findings. The conclusion is that while plasma may be preferable to whole blood when the hematocrit is very high and whole blood is preferable if the loss of red cells has been considerable or is continuing, delay in the replacement of the blood-volume deficiency is more hazardous when the latter is due to loss of plasma than when it is due to loss of whole blood.

TEMPERATURE EFFECTS

Shock due to loss of blood volume is intensified by a deficiency in the state of hydration or nutrition or by bacterial and other toxins. Successful therapy requires the correction or elimination of such factors as far as possible and the avoidance of inflicting injury by agents commonly used in the care of the shocked patient. In this connection the effect of local and environmental temperature was reinvestigated. The use of hot-water bottles, blankets, hot drinks, and a warm environmental temperature was familiar routine up to recent years. Their propriety has been reassessed in terms of the following evidence.

In experimental muscle-crush injury to the dog, gas formation, fever, and early death occurred when the environmental temperature ranged from 24 to 27° C., whereas at temperatures between 16 and 20° C. gas formation was inhibited. Death from muscle injury to one leg was reduced from 100 per cent at 28° C. to zero at 16° C. Refrigeration of a crushed leg, even at a room temperature of 28° C., resulted in survival. Refrigeration of a tourniqueted extremity before release of the tourniquet prevented death from shock. The use of ice packs applied soon after release of the tourniquet prolonged survival time. In animals dying from the injury all the blood changes were in the direction of hemoconcentration; that is, plasma loss. Shock after muscle-crush injury appears late (after some twenty-four hours) and is prevented by chemotherapy; this shock is presumably due chiefly to bacterial action. Shock induced by the release of tourniquets appears early

and is cured by volume-replacement therapy but not by chemotherapy. Hence, the protective effect of lowered local or environmental temperature is due to the vasoconstrictor action of cold, which reduces the loss of fluid into the injured extremity, to inhibition of the invariably superimposed infection, or to both. On the whole it was evident to all investigators that a hot, humid environment was a most unfavorable one for shocked animals. This is to be expected since the deleterious effects of high temperature on metabolic exchange in anoxic states is well recognized. Accordingly, the Shock Committee of the National Research Council urged that wounded men in shock be treated so as to conserve body heat, but stressed the importance of not adding to the body heat any external source of heat.

ANESTHETICS AND ANALGESICS

Among the first subjects studied in these investigations was the use of anesthetics and analgesics in shock, because of their direct bearing on clinical treatment. The intensification of the depth of shock by anesthetics had long been recognized. The avoidance of ether, nitrous oxide, and spinal anesthesia required little re-emphasis, even though the mechanism of their disturbing action, especially in the case of ether, was not altogether clear. The great convenience of intravenous barbiturates was likely to spur their widespread use in wounded men. Added encouragement of this practice had come from the observation by Seeley et al. that barbiturates inhibit lymph flow and accordingly may be regarded as a useful dampening agent against the further depletion of blood volume. Beecher subsequently confirmed this finding in burns and suggested the possible usefulness of barbiturates for this purpose. Many investigators have been impressed by the rapid precipitation of the shock state in malnourished or traumatized animals by the administration of barbiturates. Since the counterbalancing effect of barbiturates on oozing surfaces offers small compensation against this hazard, the risk attending their use in patients in deep shock was realized and emphasized by the Shock Committee. Experimental data justifying this attitude appeared from several sources, as follows:

(1) Hemorrhagic shock in dogs not medicated in any way may be reversible, even after a number of hours of severe hypotension.

(2) If barbiturates are given, especially after the shock has persisted for some hours, irreversibility is very likely to follow.

(3) A tourniquet applied to the leg of a dog for five hours does not induce deep shock, but if barbiturates are given fatal shock results. The survival rate of nembutalized rabbits in tourniquet shock was half that of non-nembutalized rabbits.

(4) The shock produced by continuous administration of adrenalin was not fatal unless the animal was under nembutal.

(5) Microscopy of peripheral flow in the exteriorized omentum of the dog in shock demonstrates the rapid onset of peripheral stagnation and loss of tone in terminal arterioles immediately after the administration of barbiturates; this effect is even more intense when ether is used.

Cyclopropane does not influence peripheral flow, and morphine affects it very slightly, although adversely. The virtually indispensable need for morphine in wounded men makes a thorough study of this effect of this drug in shock necessary. Blalock found the tolerance to bleeding unaltered by morphine. An extensive series of dogs that received a single dose of morphine (2–3 mg./kg.) before the induction of shock showed no noticeable harmful effects as compared to dogs under barbiturates (Fine et al.). On the whole, the evidence permitted the Shock Committee to rule that a single intravenous dose of morphine for the relief of pain in shock was safe and proper except in the case of cerebral trauma.

THERAPY OF IRREVERSIBLE SHOCK

From the point of view of practical therapeutics, it was widely recognized that the primary need in traumatic shock (exclusive of that due to sepsis) is an ample store of whole blood and plasma or adequate blood substitutes. With these agents the problem is solved for most patients if the volume lost is replaced in time. Further investigation was needed for an understanding of the basic disturbance in patients who fail to respond to volume therapy, either because of undue delay or because of other complicating factors. A therapeutic method was therefore sought for the state of shock showing no sustained favorable response to the reinfusion of all blood or plasma removed or lost.

To test therapeutic agents for this purpose, a technic was required that could be relied on to produce a state of irreversibility with reasonable uniformity. This was achieved by bleeding a morphinized dog from an artery connected to a bottle containing heparin and elevated to a level of 30 mm. of mercury. Bleeding stopped when the blood pressure reached this level, and stabilization of the blood pressure at this level was maintained for one and a half to four hours. Sometime during this interval the peripheral circulation began to fail and blood returned to the artery. If the peripheral vessels failed to regain tone after one third to one half of all the shed blood in the reservoir had returned, the rapid reinfusion of the remainder, with or without additional blood, failed to produce a favorable response in over 90 per cent of such animals, and death followed soon thereafter. The taking up of one third to one half of the shed blood was an almost unfailing sign of existing irreversibility.

The test of the efficacy of a therapeutic agent for the treatment of irreversible shock is unreliable unless given after irreversibility has been demon-

strated by the taking-up phenomenon as defined or by the failure of trans-fusion. Survival in response to treatment by any agent other than blood or blood substitute, even with some degree of regularity, before irreversibility has been demonstrated by either of these two conditions is not a valid test. Prolongation of survival time for less than twenty-four hours is not sig-nificant, since survival time varies considerably. With these considerations in mind, a variety of agents were tested, including whole blood, 5 per cent plasma and 25 per cent bovine albumin, 5 per cent saline solution, phys-iological saline solution in large volume, paredrine, tuamine, hypertensin, pitressin, ergotamine, 5 per cent dextrose solution, sodium succinate, sodium bicarbonate, and cytochrome C. Except for cytochrome C, substantial thera-peutic benefit was not achieved by any of these agents. Of 14 dogs to which cytochrome C was given when the first evidence of progressive failure of the peripheral vascular mechanism appeared — that is, at the onset of the taking-up phenomenon — 9 survived after all the blood had been reinfused. This substance, which has the property of releasing whatever oxygen is available for tissue consumption, deserves further study.

TISSUE ANOXIA

The anoxemia in shock is severe and progressive, so that the oxygen con-centration in auricular blood may be close to zero. Oxygen therapy, how-ever, is of no value, since even when the oxygen saturation of venous blood is increased to normal by the breathing of 100 per cent oxygen at 2 or 3 atmospheres, no noticeable improvement in the shock state is achieved. Nevertheless, it is generally recognized that the progressive disintegration of the organism in shock is due to prolonged tissue anoxia. The metabolic effects of anoxia are dealt with in the last section of this chapter.

Functional disturbances attributable to anoxia have been shown to occur in various organs. Thus, absorption of water and 5 per cent glucose from the intestine is markedly depressed, the excretory function of the liver is disturbed, and cardiac function is not entirely normal in the late stage of shock.

The effect of anoxia on the kidney has been defined with considerable precision. In contrast to most tissues, the kidney cannot compensate for decreased blood flow resulting from a fall in blood pressure by greater extraction of oxygen from the blood. The decline in excretory function parallels the decline in oxygen deprivation, and when the blood pressure falls below 60 to 70 virtually complete stoppage of excretory function occurs. The extent of recovery of renal function depends on the duration of renal ischemia; if the latter continues for more than four hours, renal failure eventually results. The renal injury in crush syndrome closely resembles that produced by incompatible blood. Sterile purified hemoglobin does not

injure the kidney, but circulating myoglobin from damaged muscle is said to precipitate hematin crystals in the tubules if the urine is acid. Since the effects of renal damage occur many hours to days after shock occurs, the renal injury in simple blood-volume loss or in muscle-crush injury does not influence the course of the shock state except insofar as the excretion of renin by the ischemic kidney into the circulation during the early phase of shock provides a stimulating or sustaining effect on the peripheral vascular tone by the interaction of renin with hypertensinogen to produce hypertensin. As the shock process progresses renin excretion stops, and hypertensinogen from the liver is either no longer available or is entirely exhausted. The vasodepressor material elaborated by the increasingly anoxic liver is then said to inflict a paralyzing effect on the tone of the arteriolar and precapillary musculature.

THE LIVER IN RELATION TO SHOCK

Since the problem in irreversible shock is one of restoring volume and velocity of flow through peripheral vessels, and since all efforts to correct the hemodynamic failure by a great variety of methods calculated to influence the circulatory apparatus directly have not succeeded, it was natural to consider the possibility that the loss of integrity of some extravascular controlling factor might be responsible. The extensive biochemical disintegration in shock renders the liver suspect. Accordingly, viviperfusion experiments were set up in which some 250 to 400 cc. per minute of circulating blood from a donor dog was delivered to the liver of a dog in hemorrhagic shock via the splenic vein and back to the donor from the arteries of the dog in shock. Viviperfusion was started at the onset of the shock state and was continued until a state of irreversibility had arrived, as judged by the standards outlined above. All but one of 14 dogs survived following return of all the shed blood. In contrast, 14 of 15 control dogs, in which the donor dog's blood was perfused into the jugular instead of the splenic vein, died. Similar results were obtained in two similar series of dogs in which the viviperfusion was started after irreversible shock had been demonstrated. The liver-perfused and jugular-perfused dogs showed a remarkable difference in response to viviperfusion. The liver-perfused dogs stopped taking up almost entirely in less than one hour after perfusion was begun, indicating recovery of peripheral vascular tone. Concomitantly, there was progressive improvement in alertness and strength, return of restlessness, and a permanently sustained blood pressure, followed by recovery. The jugular-perfused dogs, with one exception, did not stop taking up, declined precipitously, and died early. All donor dogs in all the experiments survived without injury. The donor dog's contribution to the dog in shock is an improved flow of oxygenated blood through the liver. The neutralization of toxins or their excretion by the

donor dog is not involved, since this function is available to the jugular-perfused dog as well as to the liver-perfused dog.

Collapse of the peripheral circulation in shock may therefore be considered to result from the damaging effects of anoxia on the liver. The importance of the integrity of liver tissue in the recovery from advanced shock is thus clearly established.

III. Intermediary Metabolism in Shock

C. N. HUGH LONG

Until recent years the study of shock has been conducted along three main lines: hemodynamics, the reasons for the reduced blood volume (fluid loss and so forth), and the role of toxic agents (infection and abnormal metabolites) in the genesis of the shock syndrome. While there may still be differences of opinion concerning the relative importance of primary fluid loss as compared with other factors, it is generally agreed that after injuries leading to shock there is sooner or later a striking and progressive reduction in the volume of circulating blood. Unless this is corrected the patient soon passes into the stage termed "irreversible shock," and at this time even massive transfusions with whole blood may fail to bring about resuscitation.

It is also appreciated that the time factor is of the utmost importance in the successful treatment of severely injured persons. A timely transfusion of even isotonic saline solution may be life-saving, whereas delay may bring the patient to a point where transfusion of any kind is of no avail. It may be said that the causes underlying the transformation of reversible into irreversible shock have their origin in the widespread disturbances in intermediary metabolism that follow the exposure of the tissues to a progressively diminishing blood supply. The manner in which the reduction in blood supply is brought about does not appear to alter the metabolic picture in irreversible shock, being identical so far as may be judged with that in shock arising from a variety of causes. It may also be assumed, since there is no evidence to the contrary, that the main effect of a reduced blood supply is due to the diminished quantity of oxygen available to the tissues.

Consequently, sooner or later in the events following exposure to conditions leading to shock the metabolic picture is dominated by the effects of tissue anoxia. It is on this thesis that much of the work done during the war on the intermediary metabolism in shock has been predicated. The object of such research was to obtain a clearer understanding of the nature of these metabolic changes, with the hope that methods of treatment other than those of transfusion might be obtained, and if obtained would become at least

valuable adjuvants. While it cannot be said that this hope has been achieved, the projects carried out under the auspices of the Committee on Medical Research have at least afforded some information that in the future may well serve as a better basis of treatment than the methods now employed.

THE ADRENAL CORTEX AND SHOCK

Even prior to the war, considerable work had been carried out on the role of the adrenal cortex in shock. These studies may be said to have had their initiation in the observations of Swingle, who pointed out that many of the phenomena seen in shock were identical with those found in adrenocortical insufficiency. Furthermore, adrenalectomized animals were extremely sensitive to trauma, cold, hemorrhage, and so forth — far more so than normal animals — but could be returned to a state of normal resistance by the injection of adrenocortical hormones. These facts were not disputed, but it remained to be shown that the resistance of normal animals could be improved by the use of these hormones. It might be assumed that the adrenal glands of normal persons could become exhausted following severe trauma, and if this were the case the use of additional quantities of hormone would be justified.

My colleagues and I were able to show that activation of the adrenal cortex occurs with equal rapidity and to an equal degree whether it follows hemorrhages that lead to shock or those that do not. In other words, an increased degree of adrenocortical secretion is a normal response to injury and is independent of the occurrence of shock. The methods used to determine adrenocortical activity were based on the decrease in the levels of adrenal ascorbic acid and cholesterol, an event that had previously been shown to be a consequence of an increased rate of secretion of the pituitary adrenotrophic hormone. It was also evident from these studies that the limiting factor in the response of the adrenal cortex might not be its capacity to produce hormones but the rate at which the pituitary trophic hormone was produced. When shock had been established in rats by hemorrhage, the administration of large quantities of cortical hormone was without beneficial effect. It may be concluded that while an increased quantity of cortical hormone is necessary for normal resistance to shocking procedures, it is not in itself the sole determinant of shock. Swingle also concluded that cortical hormones have little benefit in combating shock in normal dogs, a conclusion reached by Ingle in rats.

Since the problem had never been thoroughly studied in man, Lee and Rhoads conducted an extensive study of the value of adrenocortical extract in human burns. Their study was carried out on 25 patients; 13 served as controls, and the remainder received as far as possible the same treatment except that they were also given cortical extract. The use of the extract did

not significantly alter the course of events during the first twelve hours after the burn, nor did it increase the retention of plasma protein that was transfused in all cases. Finally, the complications developing in the later period after the burn, such as hyperbilirubinemia, were no less in the hormone-treated than in the untreated group.

THE KIDNEY IN SHOCK

It is well known that the blood nonprotein nitrogen rises soon after shock develops, and this has been interpreted as a consequence of renal insufficiency induced by the concomitant hypotension. As will be seen, although the alterations in protein metabolism in shock are not entirely of renal origin, the function of the kidney in these conditions has become a matter of greater import for other reasons. Early in the war the British authorities reported that men trapped in wrecked buildings for some time, particularly those who were held down by heavy objects on one or more extremities, were exceedingly liable to develop severe renal insufficiency several days after complete resuscitation from shock (crush syndrome). Later, when the extensive and early use of plasma or whole blood was introduced, with a consequent saving of many who would otherwise have died in shock, it was also observed that varying degrees of renal insufficiency developed at a time when the immediate shocking effects of trauma had disappeared.

The work of Van Slyke and his colleagues and that of Page have done much to clarify this problem. There are evidently two components that may or may not operate together to produce renal insufficiency during or after shock. The first of these is the remarkable reduction in renal blood flow that occurs when a man or animal is subjected to procedures (hemorrhage, trauma, and so forth) that reduce the blood volume. With the modern methods available for quantitative determination of renal blood flow, glomerular filtration, and tubular reabsorption, it was shown that following hemorrhage the renal blood flow was often reduced to less than 10 per cent of normal, and that this continued throughout the period of shock. Consequently the kidney may be rendered almost totally ischemic for some hours, and the question at once arises as to the degree of permanent damage, apart from the acute insufficiency that may be caused. Van Slyke and his colleagues were able to show by clamping the renal arteries in normal dogs for various periods of time that the resistance of the normal kidney to total ischemia is remarkably great. When the period of ischemia was not greater than three hours, renal function could be restored after readmission of blood. However, normal function did not return at once, and for a period of some days obvious disturbances could be found, which slowly disappeared. Periods of renal arterial occlusion greater than four hours were followed by death from uremia in four to ten days. In dogs it could also be shown that varying de-

grees of renal insufficiency were present for some time after resuscitation from shock by transfusion.

These experiments furnish an adequate explanation for at least a certain number of the cases of renal insufficiency observed in wounded men. Evidently the renal blood flow is reduced soon after severe injury, but provided the circulation is restored within a reasonable time no permanent renal injury occurs, although temporary derangements of function may be present for several days.

The reduction in renal blood flow is not the only factor concerned, however, particularly in the genesis of the severe and often fatal uremia of the crush syndrome. Both Page and Van Slyke have found at least one other possible factor — the release into the circulation of the products of red-cell hemolysis; that is, methemoglobin or other substances. Such hemolysis is particularly liable to occur after extensive muscle trauma. Under these circumstances the renal tubules become plugged with casts of material that probably had its origin in the lysed red cells. Indeed, in its effects on renal function and disease the crush syndrome closely resembles that of transfusion reactions and black-water fever.

These studies emphasize again the modern basis of the treatment of shock. This is to treat early and adequately by transfusion, so that the blood volume is restored as soon as possible. Once the secondary effects of prolonged exposure to a reduced blood volume have appeared, the treatment becomes progressively difficult and if too long delayed fails entirely.

THE LIVER IN SHOCK

In view of the numerous and important functions of the liver, it might be expected that any prolonged derangement of blood supply would have marked effects on intermediary metabolism. As a matter of fact, a reduction in blood flow places this organ in double jeopardy: it suffers along with other organs from the reduced arterial flow, but in addition there is a striking reduction in portal blood flow. In the latter case the retardation of flow in the splanchnic area brings about a marked desaturation of the venous blood entering the portal system. Normally this has an oxygen saturation of 50 to 60 per cent and constitutes perhaps as much as 80 per cent of the blood supply of the liver. In shocked animals not only is the portal blood flow reduced but the oxygen saturation may fall as low as 15 to 20 per cent. Under these circumstances the oxygen saturation in the hepatic veins may be as low as 5 per cent. Since this organ normally requires a considerable supply of oxygen for the maintenance of its functions, such reductions in blood flow and oxygen content are likely to lead to marked hepatic insufficiency. As in the case of the kidney, the permanence of the damage inflicted in shock is likely to be a function of the time of exposure to such conditions.

It is not difficult to show the acute effects on hepatic function of such reductions in oxygen supply. Winternitz and Long and later Wilhelmi and Mylon observed that the formation of urea, an obligatory aerobic process, is affected early in shock and may be reduced to 20 per cent of normal. At the same time the rate of removal of amino acids is decreased, and this, coupled with the increased delivery of amino acids from the anaerobic peripheral tissues, causes a striking rise in plasma amino nitrogen. The capacity of the liver to form glycogen from glucose and lactate is reduced. As shock continues certain essential coenzymes are released from their intracellular attachments and appear in increased quantities in the blood stream. Thus, Lamson and his colleagues found a reduction in tissue thiamine and a marked increase in serum thiamine. The alloxazine nucleotides are also decreased in the tissues, and the riboflavin content of the serum rises. The liver loses ascorbic acid, while at the same time the blood levels increase. Such effects on cellular coenzymes are also produced by subjecting the liver to anoxia, and in shock they represent the final deleterious results of a diminished blood flow.

The total respiration of the liver is decreased in shock. Thus, liver slices from rats in shock may respire at only 50 per cent of the normal rate, even though adequate oxygen is available. Addition of succinate to the medium causes a marked increase in respiratory rate, but the effects of this extra metabolism are purely ephemeral and do not bring about a synthesis of high-energy phosphate bonds, a consequence of a normal and orderly cellular oxidation. Although the use of solutions of organic acids such as succinate, with or without the addition of vitamins, has been suggested as a corrective in this stage of shock, there is little evidence of beneficial results.

Most of the metabolic disturbances observed in the livers of shocked animals can be reproduced by subjecting the normal liver to various periods of anoxia. Engel, Wilhelmi, and Russell were able to devise a preparation in the rat in which the sole source of blood supply was through the hepatic artery. Under resting conditions and for some hours such a liver behaves in a normal manner. The hepatic artery was clamped for periods of fifteen minutes to two hours and then released. The behavior of the liver after varying periods of recovery was followed by noting its capacity to reduce the high level of amino nitrogen present, as well as its total respiration. It was found that the liver of the rat would completely recover after total anoxia for fifteen minutes. Longer periods resulted in a progressively greater degree of hepatic insufficiency, as judged by the above criteria. In contrast to skeletal muscle and the kidney, the capacity of the liver to resist anoxia is significantly less, but it is of course not as limited as that of nervous tissue.

The value of maintaining an adequate blood supply to the liver during shock is strikingly shown by the experiments of Fine and his colleagues. They devised an ingenious technic by which the blood flow to the liver could

be maintained at almost normal levels while that to the rest of the animal was reduced to levels that produced fatal shock. This involved a cross circulation from a donor animal to the splenic vein (or to any other vein). When the blood supply to the liver was maintained in this way practically all the dogs survived, whereas those in which similar transfusions were made into the jugular vein died. Even if irreversible shock has developed before the separate liver circulation is established, a marked increase in survival time is found. Such animals are, however, extremely likely to die from complications introduced as a result of damage to the cerebrum by the severe anoxia.

INTERMEDIARY METABOLISM IN SHOCK

The majority of the numerous metabolic disturbances found in shock are to be attributed to the progressive tissue anoxia. The reduced blood flow steadily forces the tissues to depend for their energy requirement to a greater and greater degree on anaerobic processes of metabolism. There is obviously a limit to which this can be carried without cellular derangement to the point of irreversibility. Furthermore, the various tissues differ greatly in their capacity to continue on a more or less anaerobic basis. The skeletal muscles have such a capacity to a marked degree; indeed, the essential features of their normal metabolism are of this kind. The kidney normally reduces its blood flow to an extreme degree following acute reduction in the blood volume. It may tolerate severe degrees of ischemia for a surprising length of time, but eventually irreparable damage ensues. Apart from the nervous tissue, the liver appears to have the least tolerance to ischemia of all organs so far studied, and although hepatic insufficiency is only rarely the direct cause of delayed death after injury, the relative incapacity of this organ may be a critical factor in determining the outcome.

It is now known that the energy derived from oxidative processes is utilized by cells only after its preliminary transformation into high-energy bonds found in certain types of cellular components. Among these the most important are creatine phosphate and adenosine diphosphate and triphosphate. The phosphate groups of these compounds require the absorption of large quantities of metabolic energy for their formation. This energy is in turn obtained from contemporary oxidation. Once formed, such group energy may be directly used for cellular function. Meyer and Potter have shown in shocked rats that there is a loss of such energy reservoirs from the tissues, and they associate the inability of the cells of such animals to function normally with the failure of the oxidative metabolism to provide a normal rate of replenishment. In effect these observations are in keeping with those on certain coenzymes, which under ordinary circumstances are maintained in cells in a phosphorylated form by the continuous provision of metabolic energy.

As one surveys the work that has been carried out so far on intermediary metabolism in shock, it becomes apparent that the deviations from metabolism observed are of two kinds: those associated with the vigorous compensatory mechanisms promptly evoked by trauma, blood loss, and so forth; and those characteristic of progressive tissue anoxia. Prominent among the former are the activity of the sympathetic nervous system and the release of epinephrine, whose effects on carbohydrate metabolism hardly need description. There is now evidence of activation of adrenocortical secretion, which influences the inorganic as well as the organic pattern of the blood and tissues. None of these effects are to be regarded as due to shock, since they occur before it develops, but the changes in metabolism that they bring about may and do persist when this occurs.

The metabolic changes characteristic of progressive tissue anoxia are numerous and vary from organ to organ depending on their metabolic characteristics. Not one of these changes (namely, increased plasma acid and lactic acid, decreased carbon dioxide content and so forth) is in itself a reliable criterion of irreversible shock. It appears that the extent to which the circumstances that lead to them have progressed is more important than the magnitude of the change. The cause in all cases so far examined is the failure of the tissues to adapt themselves to a progressively severer degree of anoxia, the culminating point of which is cellular disorganization and death. The failure of a restored blood volume effected at this time to correct these metabolic deficiencies simply means that the cellular mechanisms for uniting the blood oxygen with the hydrogen and carbon of the metabolites have ceased to function.

HYPOTENSIVE FACTORS

A theory has been persistently advocated for many years to the effect that the hypotension and reduced blood flow in shock are perpetuated by the release into the circulation of vasodepressor substances. These substances may either be derived from the presence of bacteria that so frequently invade traumatized areas or arise as a consequence of the liberation from the damaged or anoxic tissues of normal cellular components that exert a vasodepressor action. The possible relation of bacterial products to shock is discussed elsewhere.

Shorr and his colleagues have presented the case for the presence in the circulation in shock of certain physiological vasodepressor materials in increased amounts. Their observations are based on the behavior of the capillary network during and following the induction of shock by hemorrhage. In the first place, this network exhibits an increased reactivity to epinephrine, which is later succeeded by irresponsiveness to this agent. There is evidence that in the first phase vasoconstrictor substances are liberated

from the kidney or elsewhere as part of the compensatory response to the blood loss, but as hypotension proceeds the picture becomes dominated by the accumulation of vasodepressor substances. These are regarded not as abnormal constituents of the blood but as continually produced by the tissues and inactivated by the liver. In shock, liver function is so depressed that it fails to preserve the physiological levels of vasodepressor material. The amounts in the blood increase to the point where damage to the circulation occurs.

This is a new approach to the problem of blood-pressure regulation by humoral agents. It should be pointed out, however, that it still assumes that progressive tissue anoxia due to reduced blood flow is the agent in the production of hepatic insufficiency on which the accumulation of vasodepressor material depends. Indeed, so far it has not been shown that shock can be initiated by the release of cellular constituents into the circulation. These may be released by subsequent tissue anoxia and add their cumulative effect to the circulatory deficiencies.

CHAPTER XXVIII

THE HISTORY OF PLASMA FRACTIONATION

EDWIN J. COHN

I. INTRODUCTION

 B LOOD and blood derivatives have been used more extensively in this war than in any previous time. Colloidal particles of large size were employed in combating shock, through their action of drawing water into the blood stream, during World War I. The colloid employed was gum acacia, and controversies have persisted ever since regarding tissue deposition following intravenous injection of large amounts of this and of other suggested blood substitutes.[1] Although the principle of effecting a re-equilibration in water and electrolytes, between the blood and interstitial fluids, by the injection of colloidal material to combat shock was thus established in earlier wars, procedures had not been developed for making available adequate amounts of the ideal colloid.

The components of the blood that normally control its volume are the plasma proteins. The physical properties of these native plasma colloids normally lead to their retention, at a given level, within the blood stream and to their distribution, as well as that of water and electrolytes, between blood and tissues.

Among the plasma proteins the albumins are responsible for roughly 80 per cent of the force per unit area, osmotic pressure, which determines the exchange of water across membranes. The red blood corpuscles, which are responsible for the respiratory function of the blood, do not contribute directly to the forces that hold water in the blood stream. It is, however, "important to recognize that recovery of tissues from the state of shock requires restoration of oxygen transport to the tissues by the circulation; in other words, adequate circulation of hemoglobin. A normal total blood flow or cardiac output does not provide normal oxygen transport if the hemoglobin concentration is low. As a measure of oxygen supplied to the tissues, one may use the total arterial oxygen transport, which is actually the cardiac output times the total oxygen content of the arterial blood. This determines the total amount of oxygen brought to the tissues per unit of time.

[1] See Chapter XXIX. In the preparation of this chapter, which has been carefully documented in the interest of those who will continue to use these processes and products in the postwar world, I have been immeasurably aided by Drs. Scatchard, Janeway, Oncley, and Edsall.

"In shock due to skeletal trauma or hemorrhage, the reduction in blood volume is regularly associated with *hemodilution* (1). By giving whole blood in such cases, both the blood flow and the amount of hemoglobin which is circulated are increased. By giving albumin (or plasma), the arterial oxygen transport is restored to normal only if the cardiac output is correspondingly increased above normal. . . . In brief, after treatment with albumin or plasma, such a patient has recovered from shock, but is still suffering from acute anemia" (2).

WHOLE BLOOD

The need for whole blood for a combat army was clearly recognized by the advisors of the armed forces, who began to gather in Washington in 1940 (3). The life of the red blood cell within the body is, however, roughly three months, and in the blood bank, even under the best conditions that have been developed during this war,[2] only about a third of this.

It was thus necessary to recommend to the armed forces, in addition to whole blood, stable blood derivatives or substitutes, supplies of which could be accumulated in advance, against a future need, at an unknown time, and in an unknown theater of operations.

During the spring of 1940, when the German offensive had begun on the Continent, and before the fall of France, the urgent request was made to the recently reorganized Committee on Transfusions of the National Research Council, of which the late Walter B. Cannon was chairman, for 300,000 transfusion units. Representatives of the American Red Cross were called in conference. This may be considered the beginning of planning for the blood-donor service of the American Red Cross, although the program was not put into effect until the next fall and, at the time, 300,000 donations seemed like an almost impossibly large request. That the American Red Cross would succeed in routinely collecting this much blood in a month was inconceivable at that time.

ANIMAL PROTEINS

Immediately following this conference in Washington, Dr. Walter B. Cannon and Dr. David Edsall, then Chairman of the Medical Advisory Committee of the American Red Cross, called on us to ask whether we would undertake an investigation to determine whether the plasma of animals could be made safe for human transfusion. The experiments of Dr. Owen Wangensteen of the University of Minnesota (4, 5) were brought to our attention. We undertook to do what we could and to attack the problem in

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[2] See Chapter XXX.

our laboratory, provided adequate clinical testing facilities were made available. We pointed out that there was evidence in the literature that, of the plasma proteins, the albumins, with their great effect on osmotic pressure (6) and therefore on the control of blood volume, which rendered them important for shock, were somewhat less antigenic (7) than were the globulins, and when antigenic produced sensitization more slowly (8), but that no adequate methods were available for the separation of albumins of adequate purity in the large amounts indicated.

Fractionation of Animal Plasma

New methods for the fractionation of plasma in alcohol-water mixtures, at low temperatures and controlled pH, protein, and salt concentration, were therefore rapidly developed during the spring and summer of 1940. These were based on previous studies on the solubility of amino acids, peptides, and proteins in such systems (9). The theory that even the closely related protein molecules in a multicomponent system, such as a body fluid or tissue extract, can be separated in a system of five or more variables, each under precise control, was at once applied to the fractionation of bovine plasma. In this system, salts, such as ammonium sulfate, that have in the past generally been employed as protein precipitants (10), were replaced by volatile water-miscible organic liquids. Thus, ethyl alcohol has been employed as the protein precipitant in many of these separations. Its concentration, however, has rarely exceeded 25 per cent, and at one point only in our process has it reached 40 per cent by volume.[3] The temperature was maintained at 0° C. or lower so as to minimize the effect of alcohol in denaturing proteins at ordinary temperatures. Thus, the temperature was maintained close to the freezing point of the solutions until a temperature of $-5°$ C. could be reached in 15 per cent alcohol. This low temperature has generally sufficed to yield satisfactory protein preparations of high purity. In this system of fractionation [4] the pH of the alcohol-water mixture was adjusted and accurately maintained by buffers of known ionic strength for each operation. The protein concentration was maintained sufficiently dilute to minimize the interactions between protein molecules, but as concentrated as possible in order to render economical the construction of plants for the large-scale processing of plasma. Salt concentration was maintained as low as possible so as to render unnecessary dialysis of the purified products that were separated and — with the exception of the water-insoluble lipoproteins that are denatured by freezing — dried from the frozen state by the same methods as those employed in drying plasma.

[3] Values of volume per cent and pH refer to the same systems at 25° C.
[4] Described in greater detail in Section II.

Human Plasma

Methods for drying proteins from the frozen state to yield undenatured products of great stability were developed from earlier observations during the period between the two world wars (11). These methods had been applied successfully to the drying of human plasma, and studies were available demonstrating the value of such products, reconstituted with a satisfactory diluent, in the treatment of shock (12). It was thus apparent that a satisfactory, stable blood derivative could be prepared well in advance of need and stores of it accumulated wherever military considerations demanded. One was therefore forced to conclude in 1940 that "it would be a mistake to delay much longer a set-up which would provide human blood from which plasma can be processed and . . . the logical thing to do is to use the dried plasma . . . and await development of experience with bovine and equine plasma and such substitutes as may be developed synthetically." [5]

The plasma dried from the blood collected by the American Red Cross, recommended to the armed forces by the National Research Council, has yielded one of the very satisfactory blood derivatives used in this war.

Fractionation of Human Plasma

By midsummer of 1940 it had become certain that a system for the fractionation of plasma had been developed capable of yielding not only albumin, but also the other components of plasma of different molecular characteristics and physiological functions (13). It had also become apparent that the diverse proteins of human plasma might have advantages in therapy when separated and concentrated and employed in clinical conditions related to these functions.

Although studies on the use of bovine serum albumin to determine its safety, as well as its value in therapy, continued,[6] it appeared "likely that research will show that it will be impossible to use bovine or equine plasma and that we must depend upon human plasma" [5] in the immediate future. The first fractionations of human plasma were carried out by Dr. S. H. Armstrong, Jr., in August 1940, in the Department of Physical Chemistry, Harvard Medical School, on plasma purchased from a few professional donors. The results were reported to Dr. DeKleine of the American Red Cross on the occasion of his visit to Boston in the fall of 1940 to stimulate interest in the creation of a blood-collection center in Boston in connection with the national Red Cross program, which was then beginning, and at his request to representatives of the Navy in Washington on December 21, 1940.[5]

[5] Memorandum for Adm. McIntire from C. S. Stephenson, Cmdr. (MC), U.S.N., of a conference with Dr. DeKleine of the American Red Cross and Dr. Edwin J. Cohn on December 21, 1940.

[6] Chemical developments are discussed in Section III, and the immunological and clinical studies in Chapter XXIX.

The Navy's interest in the fractionation of human plasma followed. Its special concern was the development of a safe, stable, compact blood derivative, immediately available without reconstitution for emergency use, for the treatment of shock and burns.

Normal Human Serum Albumin

Albumin not only has the greatest osmotic efficiency, but is present in the largest amount, and is the most soluble, the least viscous, and the most stable of the plasma proteins. It was possible to state by the end of 1940:

The preparation of large amounts of relatively pure albumin is now possible. . . . The albumin remaining in solution in 40 per cent ethanol at pH 5.5 at — 5° C. is largely precipitated from this solvent at pH 4.4–4.8, as is also the small amount remaining in solution at this temperature by concentration at — 15° C. Albumin, both human and bovine, has been prepared by this method and is pure both electrophoretically and in the ultracentrifuge, and there is practically no upper limit to the amounts of this material (as of fibrinogen and the γ-globulins) that can readily be made available.

The albumin precipitable from 40 per cent ethanol is readily dried and is a colorless white powder, free of reducing substances and indeed of other organic and inorganic molecules for which we have thus far tested. It readily dissolves in water, yielding clear solutions even at concentrations greater than 25 per cent. . . .

Although albumins may be lost from the circulation more readily than certain globulins, their smaller size and greater net charge lead to greater osmotic effects. The high osmotic pressure, low viscosity, and great stability of albumin solutions would appear to render them the most useful of the plasma proteins for the treatment of some, but not necessarily all, conditions associated with diminished plasma volume. (14, pp. 412–414.)

In the interest of making available larger amounts of serum albumin, and other components of human plasma, collection of blood by the American Red Cross began in the Outpatient Department of the Peter Bent Brigham Hospital on February 21, 1941, with 19 volunteer donors.[7] Weekly collections of blood from approximately this number of donors followed. The first human serum albumin preparation from this blood (HA VIII–XII) was released for clinical trial (as HA–1) on April 27, 1941.[8]

Facilities for the preparation of larger amounts of human serum albumin were made possible by a grant on April 5, 1941, from the National Research

[7] Following the conference referred to above, the American Red Cross arranged, in February 1941, to have the Boston Metropolitan Chapter organize a temporary donor center under the direction of Dr. Carl Walter in the Peter Bent Brigham Hospital until such time as the Boston Metropolitan Chapter organized its blood-donor center.

[8] The maximum amounts of albumin that could be prepared in our — 5° C. thermostats, with cup centrifuges, were so small that the yields from four or more preparations, made in as many weeks, were combined into a single preparation before sterile filtration.

Large-scale fractionation of human plasma proteins.
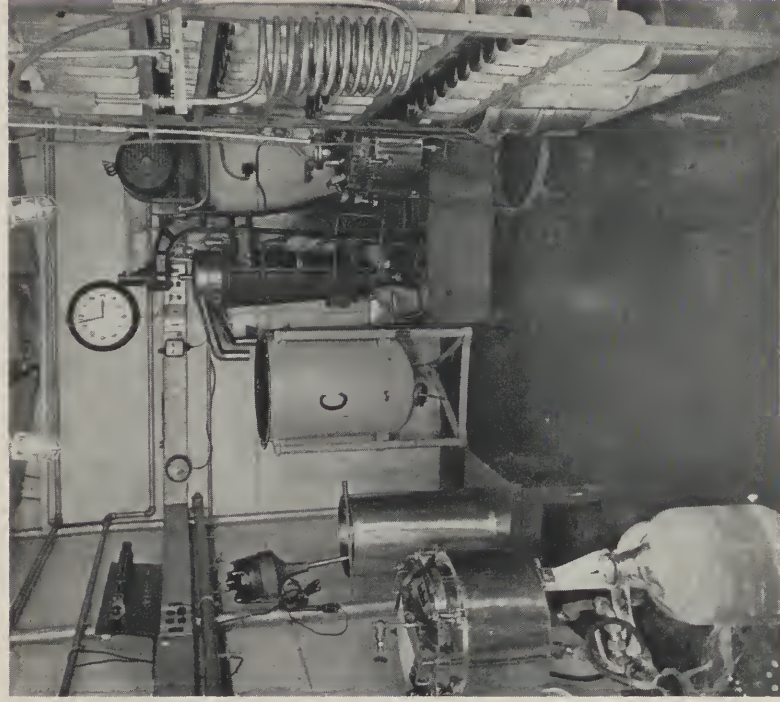
Harvard pilot plant.
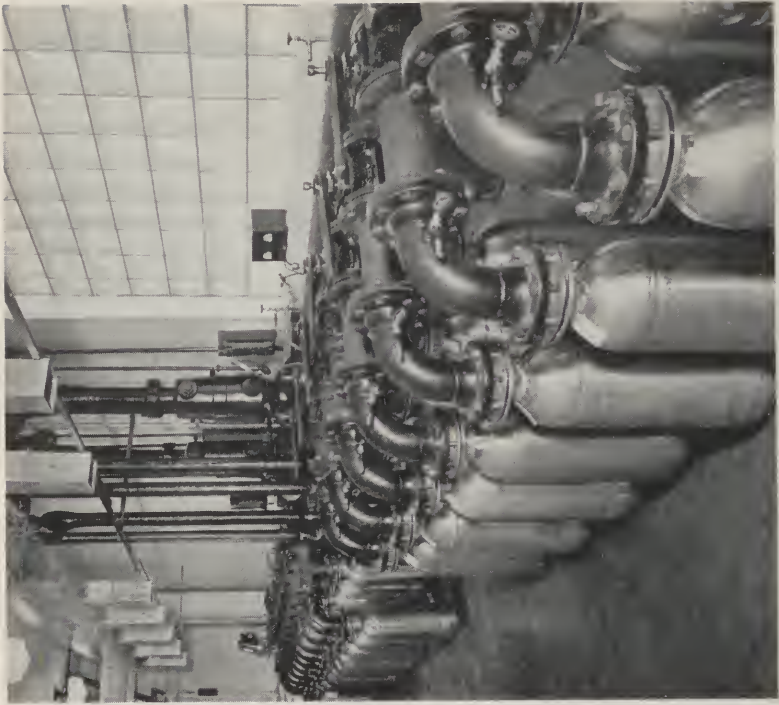
Industrial plant.

FIGURE 45a. Low-temperature fractionation room.

Large-scale fractionation of human plasma proteins.

Industrial plant.

Harvard pilot plant.

FIGURE 45b. *Drying proteins from the frozen state.*

Council "for processing human plasma into its component protein fractions." This included a grant from the fund placed at the disposal of the Committee on Medicine, National Research Council, by the American College of Physicians.[9] Construction was thus made possible of the Harvard Pilot Plant for Plasma Fractionation (Fig. 45A and B), in juxtaposition to the Ultra-centrifuge Laboratory at the Harvard Medical School. The first preparation completed in it (HA–4) was released for distribution on July 9, 1941. Fractionation of plasma has been continued uninterruptedly in this plant ever since. For five years, therefore, each advance in research has been tested in the Pilot Plant before being recommended in directives for large-scale production in other laboratories.

Collection of blood by the American Red Cross and completion of the Harvard Pilot Plant made possible the preparation of adequate amounts of normal human serum albumin so that clinical appraisal could begin. The voluntary collaboration of Dr. Elliott Robinson, Director of the Massachusetts Antitoxin and Vaccine Laboratory, in carrying out the sterility and safety tests and releasing the material for clinical trial, and of the late Dr. Soma Weiss, Professor of Medicine and Physician-in-Chief at the Peter Bent Brigham Hospital, in arranging for the clinical trial of albumin, led to a vigorous, well-co-ordinated program's being well under way by the summer of 1941. The intimate, day-by-day collaboration of the chemical group in the Department of Physical Chemistry which included Drs. J. T. Edsall, R. M. Ferry, J. L. Oncley, J. D. Ferry, L. E. Strong, W. L. Hughes, Jr., S. H. Armstrong, Jr., and M. Melin, with the staff of the Massachusetts Antitoxin and Vaccine Laboratory, and with the clinical group that Dr. Weiss brought together [11] and which included Drs. C. A. Janeway, O. T. Bailey, J. T. Heyl,

[9] ". . . The Administrative Committee of the National Research Council approved an appropriation of the sum of $5,000 to Harvard University Medical School for support of investigations under the direction of Dr. Edwin J. Cohn. This grant was made on recommendation of the Executive Committee of the Committees Advisory to the Surgeons General and was designated as support of the research-program, which was briefly described as 'a project for processing human plasma into its component protein fractions.'

"In accordance with the terms of the contract between the Health and Medical Committee of the Federal Security Agency and the National Research Council, forty per cent (40%) of the grant will become immediately available and the balance will be paid in monthly installments. It is hoped that the project will be completed by June 30, 1941.

"An additional $5,000 (making a total allocation of $10,000) has been obtained by a grant from the fund placed at the disposal of the Committee on Medicine, National Research Council, by the American College of Physicians. This sum becomes immediately available. . . ." [10]

[10] Letter of April 5, 1941, to President James B. Conant, Harvard University, from Dr. Lewis H. Weed of the Division of Medical Sciences, National Research Council.

[11] The clinical group included L. M. Woodruff, then Lieut. (jg), M.C.–V(G), U.S.N.R., later Commander, M.C., U.S.N.R., and S. T. Gibson, then Lieut. (jg), M.C.–V(G), U.S.N.R., later Commander, M.C., U.S.N.R., assigned to our laboratories by the U. S. Navy.

and C. v. Z. Hawn, has continued throughout the war. Moreover, this collaboration is continuing into the peace, since the fractionation of human plasma collected for the blood and blood derivatives program of the Massachusetts Department of Public Health was carried out during the spring of 1946 in the Harvard Pilot Plant during the period that the new laboratory for plasma fractionation was being completed as an addition to the Massachusetts Antitoxin and Vaccine Laboratories.

Natural growth led to Dr. George Scatchard of the Research Laboratories of Physical Chemistry of the Massachusetts Institute of Technology associating his laboratory with ours in June 1941, in physicochemical studies of the products being developed. During this period, at our request and without financial commitments of any kind, the Armour Laboratories in Chicago built a pilot plant for the fractionation of bovine plasma. During this summer also, Dr. Joseph Stokes of the Children's Hospital of Philadelphia, hearing of our separation of γ-globulin, came to Boston to offer his collaboration in the study of its value in measles prophylaxis. In the fall he wrote, "On the agenda which Francis Blake has just sent to me for the meeting of the Board for the Investigation and Control of Epidemic Diseases in the Army next Friday, November 28th, one of the items is a discussion of the possible use of human globulin (Cohn) in the passive immunization against measles."

Within the Department of Physical Chemistry, advances in research ran parallel with developments in the Pilot Plant and in the clinic. Dr. L. E. Strong had become Associate Director of the Pilot Plant. Control of the molecular size and shape of the separated and purified products had been organized by Dr. J. L. Oncley, Director of the Ultracentrifuge Laboratory, and control of uniformity by measurement of electrophoretic mobility by Dr. S. H. Armstrong, Jr., who had assumed direction of the Tiselius Laboratory. Studies on the substances and conditions determining the properties of the blood clot had been begun by Drs. R. M. Ferry, J. D. Ferry, and J. T. Edsall. The method for the crystallization of serum albumin, human and bovine, from alcohol-water mixtures was developed in collaboration with Dr. W. L. Hughes, Jr., following a chance observation in June, 1941.[12]

Routine production was thus assured, not only by the reproducibility of the fractionation process but by the careful control of the physical properties of the products of plasma fractionation. Every preparation of these new plasma products that has been made available, either for clinical studies or

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In 1942 when Navy contracts for albumin had been made E. J. Klein, then Ensign, HC, U.S.N., later Lieut., HC, U.S.N., and Harold L. Taylor, then Ensign, A–V(P), U.S.N.R., later Lieut., H–S, U.S.N.R., were assigned to the laboratory, where they remained continuously throughout the war. Later, when surgical studies had begun under the direction of Dr. F. D. Ingraham, E. A. Bering, Jr., then Lieut. (jg), M.C.–V(G), U.S.N.R., later Lieut. Commander, M.C., U.S.N.R., was assigned.

[12] See Section III.

for use by the armed forces, has been subjected to the most precise control as a purified chemical substance and used in animals and then in man before being released.

The newly reorganized Subcommittee on Blood Substitutes of the National Research Council was informed on July 18, 1941, as follows:

The unit at Harvard is making 500–800 grams of human albumin a week from blood supplied by American Red Cross donors. One thousand three hundred grams of albumin are available or have been distributed, and reports from 375 grams received. The preparation of 4000 grams which had been requested by the Committee on Blood Substitutes should be completed about August 1, 1941. . . .

The administration of human albumin intravenously appears to have led satisfactorily to pronounced drawing of fluid into the blood stream and to the raising of the blood pressure. In only one case was any reaction noted, and this was in the administration of albumin prepared from dried plasma . . . (from containers broken in processing) and reworked at Harvard. The color of this preparation was somewhat darker than comparable preparations containing the same amount of globulin, although it is by no means certain as yet that this is significant. The report on this case . . . characterized the reaction as mild, following the administration of 60 grams of protein to an individual who had lost 1080 cc. of blood. The symptoms noted were transient lumbar pains and a rise in temperature to 99.8°. In no other case was any reaction noted. . . .

The following researches on the various components of plasma and their properties were reported to be in progress:

Studies on Osmotic Pressure of the different proteins isolated from both bovine and human plasma are being carried out under the direction of Professor George Scatchard of the Department of Physical Chemistry at the Massachusetts Institute of Technology. Both freezing point and osmotic pressure measurements will be undertaken on the different fractions of plasma. This work should reveal the amount of water each species of protein should draw into the circulatory system.

Studies on the Globulins: Whereas it is probable that none of the globulins exert as large osmotic pressure — and therefore are as important in the treatment of shock — as the albumins, the osmotic effects of those of the largest molecular weight and the most nearly neutral isoelectric points are very small indeed. Globulins of this class move in the electric field with the smallest mobility and are known as γ-globulins. Their great importance in immunology and the value of the very large amounts of these fractions which will become available in the fractionation of human plasma as substitutes for convalescent serum must not be overlooked. Dr. Robinson is especially concerned with this aspect of the problem.

The distribution of the globulins, of fibrinogen and of the albumins of plasma as estimated by electrophoretic analysis is tabulated below. . . . The amount of each of these proteins that is separated in the six fractions on the basis of which our plasma fractionation procedure is now carried out, is also tabulated. Fraction I is largely fibrinogen. Fraction II is rich in the large molecular weight components of the γ-globulin complex and Fraction III in the smaller molecular weight γ-globulins. Prothrombin is distributed between Fractions II and III and is being further purified by fractional dialysis and isoelectric precipitation.

The α- and β-globulins are concentrated in Fractions III and IV and Fraction IV also contains appreciable amounts of albumin, which are recovered upon reprecipitation of the globulins. It is not yet certain whether the albumins separating respectively in Fractions IV, V and VI, are identical. Investigations to determine this as well as to study further the proteins known to possess important physiological functions are in progress.

Studies on Prothrombin are being carried out in our department by Dr. J. L. Oncley with the aid of the ultracentrifuge, which reveals the presence of the large molecular weight γ-globulin component with which it is precipitated in Fraction II. Prothrombin itself is not a γ-globulin nor a large molecular weight component, being a small globulin of isoelectric point near pH 5.2, and studies on its further purification from Fractions II and III will be subsequently reported.

Studies on Fibrinogen are in progress in order to determine the conditions for the preparation of this protein in a purified and stable state. The investigation is being carried forward by Dr. R. M. Ferry of this department.

Studies on Fibrinogen Plastics: Fibrinogen which has been prepared as a plastic by Drs. J. D. Ferry and W. L. Hughes in this laboratory is being implanted in animals and the resultant tissue reactions studied at the Peter Bent Brigham Hospital by Drs. O. T. Bailey and R. Ford. A series of plastics of widely differing hardness and elasticity are being employed in order to determine the effect on rate of solution in the tissues, extent of tissue reactions, and character of the cellular response to the plastics which is produced by varying several factors in the preparation. Since these plastics can be prepared from protein of the same species as that in which they are to be used, they may have significant value for surgical as well as other purposes.

Studies on Cholesterol, Carotene and Vitamin A have revealed that unlike urea, sugars and most ordinary reducing agents, these substances are not readily removed from the plasma by dialysis against water or alcohol-water mixtures. The study of their distribution in the various plasma fractions has been undertaken by Drs. Allan Butler and Nathan Talbot of the Department of Pediatrics at the Harvard Medical School. Preliminary results are recorded in Table II.[13] The distribution suggests that these substances are related to the β-globulin; that is to say, the same fraction which is elevated in persons with lipoid nephrosis, cirrhosis and other diseases associated with lipemia.

Studies on the Iodine Distribution of Plasma are being carried out by Dr. W. T. Salter of the Thorndike Memorial Laboratory of the Boston City Hospital and reveal that the protein to which the iodine appears to be bound in plasma is associated rather with the albumins than the globulins. Concentration of the protein to which the iodine is bound should facilitate considerably the study of the nature of the iodine-containing components of human plasma.

Investigations of the distribution of . . . metals known to be combined with proteins in the blood, such as copper, zinc and iron, are planned and will be subsequently reported, as will studies upon the hormones and other physiologically important components of human plasma for which methods of bio-assay are

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[13] Table I in this chapter. In the report of July 18, 1941, Table I recorded early clinical results.

Table I

Protein Composition of Normal Human Plasma

	Approximate Distribution of Proteins		Proportion of Component Separated by Ethanol-Water Mixtures into Fractions					
			I	II	III	IV	V	VI
	Gm./l.	%	%	%	%	%	%	%
Total protein	65	100	7	11	8	25	45	4
Fibrinogen	4	6	80	10	5	5	0	0
γ-globulin	8	12	10	50	35	5	0	
Prothrombin			5	30	50	5	0	
β-globulin	8	13	2	18	35	40	5	
Cholesterol	*		0	14	33	46	0	1
Carotene			0	10	48	32	0	
Vitamin A †								
α-globulin	5	7	0	1	3	90	5	1
Protein-bound iodine	‡		2	2	3	33	56	4
Albumin	40	62	1	4	4	15	70	6

* 1.56 gm. of cholesterol is bound by protein in 1 l. of plasma.
† Determinations not yet complete.
‡ 52 γ of iodine is bound by protein in 1 l. of plasma.

available. We feel keenly that these should now be studied in the large amounts of human plasma fractions becoming available as by-products of the preparation of human albumin for transfusion in shock.[14]

Following the organization of the Committee on Medical Research, we were asked and agreed to continue "chemical, clinical, and immunological investigations on human and bovine serum albumin" under contract with the Office of Scientific Research and Development, commencing August 22, 1941. Until this time research had been financed by grants from the Rockefeller Foundation and from funds of Harvard University, including a grant from the Proctor Fund, and the grant from the National Research Council for the construction of the pilot plant.[15] Study of fibrinogen and thrombin, the immune globulins, and products of plasma fractionation other than albumin were continued with these funds.

The support of the Committee on Medical Research made possible an expansion of the program for the preparation and testing of human serum albumin. In order to prepare larger amounts than could be processed in the pilot plant, a contract was made between the Office of Scientific Research and Development and the Armour Laboratories during the fall of 1941 for the production of normal human serum albumin, according to our processes, from plasma collected at the Samuel Deutsch Convalescent Serum Center, Michael Reese Hospital, Chicago. The Armour Laboratories were selected because of their previous collaboration in the preparation of bovine serum albumin. The first human serum albumin prepared under this contract was ready for clinical trial on January 26, 1942.

Meanwhile, war had come on December 7, 1941. The next day, Dr. A. N. Richards, as Chairman of the Committee on Medical Research, telephoned asking whether any human serum albumin could be made available to be flown to Pearl Harbor at once. Every bottle in Boston was forwarded that night, as requested, to Dr. I. S. Ravdin for use under his direction at Pearl Harbor. The albumin was administered with striking results to a few men who were suffering from shock and hypoproteinemia subsequent to severe burns. These results, supplementing those that had been reported by the clinical group in Boston (15, 16) and by the clinical groups co-operating in other cities,[17] were reviewed at a meeting of the National Research

[14] Report to the Subcommittee on Blood Substitutes, Division of Medical Sciences, National Research Council, July 18, 1941. *Bull. Blood Subst.*, pp. 93, 98–101.

[15] In addition to the grant mentioned above there was one for $1500 for the "investigation of animal blood proteins as substitutes for human plasma; preparation of protein-fractions, with altered or denatured proteins." [16]

[16] Letter of February 11, 1941, to Dr. Edwin J. Cohn from Dr. Lewis H. Weed of the Division of Medical Sciences, National Research Council.

[17] The committee consisted of Drs. Soma Weiss, Alfred Blalock, R. F. Loeb, I. S. Ravdin, and Owen Wangensteen.

The clinics co-operating were: Atlanta, Ga., Grady Hospital; Baltimore, Md., Johns Hopkins Hospital; Boston, Mass., Beth Israel Hospital, Boston City Hospital, Massachu-
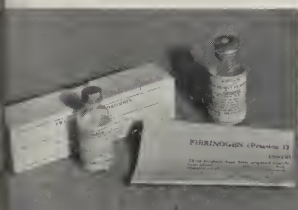
Fibrinogen and Thrombin
Fraction I and Fraction III–2
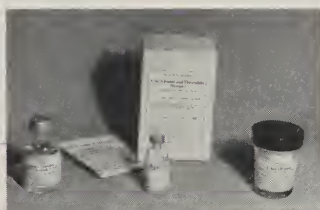
Fibrin Foam and Thrombin
Fraction I and Fraction III–2
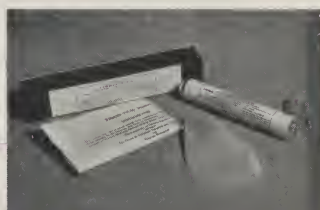
Fibrin Film
Fraction I and Fraction III–2
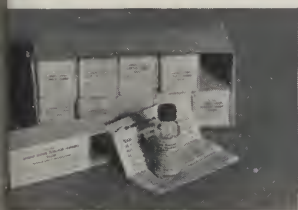
Serum γ-Globulin
Fraction II
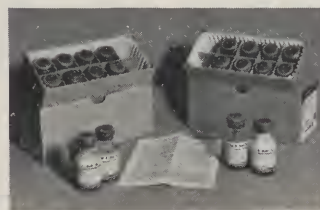
Isoagglutinins
Fraction III–1
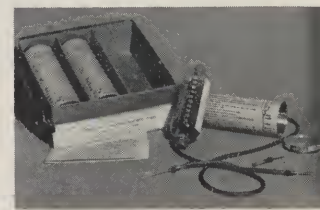
Serum Albumin
Fraction V

FIGURE 46. *Products from fractionation of human blood plasma.*

Ethanol 25 %
Decanol 0.1%
Γ/2 0.1
p.I 5.5

Human
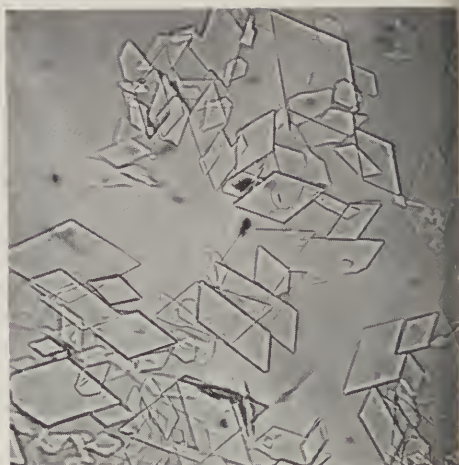
Ethanol 10 %
HgCl₂ 0.01%
Γ/2 0.05
pH 5.5
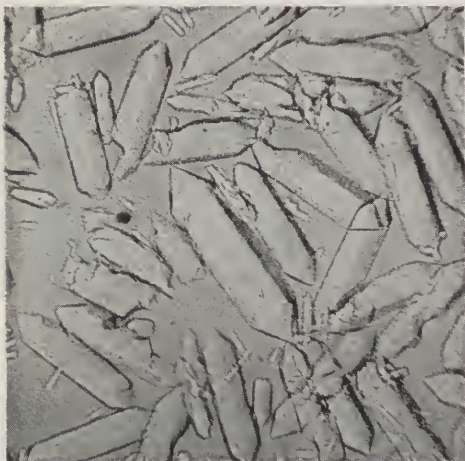
Ethanol 35 %
Γ/2 0.4
pH 5.5

Bovine
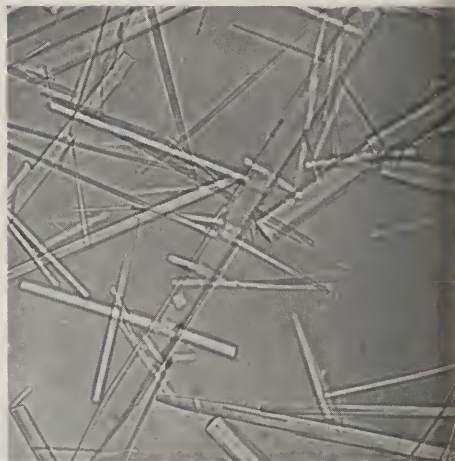
Ethanol 15 %
Γ/2 0.02
pH 5.1

FIGURE 49. *Crystalline serum albumins.*

Council on January 5, 1942. At this meeting data on 87 cases treated with albumin were summarized by Drs. S. Weiss and C. A. Janeway and Lieuts. (jg) Woodruff and Gibson (MC), U.S.N.R., and normal human serum albumin was recommended to the armed forces (Figs. 46 and 47).

NAVY CONTRACTS FOR NORMAL HUMAN SERUM ALBUMIN

Following this recommendation the Surgeon General of the Navy authorized contracts to be made for the production of normal human serum albumin in commercial firms and asked whether we would accept responsibility for the control of production. Personnel from these firms came to the Harvard Pilot Plant for training in accordance with a letter dated March 12, 1942, from Surgeon General Ross T. McIntire stating "that the technicians who are to be in charge of the fractionation of albumin in the contracting laboratories shall be sent to the Harvard Fractionation Laboratory for such instruction as will be specified by Dr. Cohn or his designated representatives" during the period that the new low-temperature commercial production laboratories were being built in New York, New Jersey, Pennsylvania, Michigan, Indiana, and California. Production of serum albumin in these plants, and in the plant subsequently built in Texas for the processing of the blood collected by the American Red Cross in the Southwest, is graphically represented in Figure 47.

During this period we were instructed to discontinue clinical testing of human serum albumin, since its value and uses were considered to have been demonstrated and the small, compact emergency unit was in demand by the Navy. From June 1942 to July 1943 — that is to say, until commercial production was well under way — the Harvard Pilot Plant, operating in three shifts twenty-four hours a day with OSRD(CMR) funds, delivered to the Navy approximately twenty-five hundred bottles of normal human serum albumin. The far larger amounts of Red Cross blood that were then being processed to yield albumin for the armed forces rendered it essential that all the other products of plasma fractionation be conserved to be made available for such therapeutic uses as might be demonstrated.

The specifications of the Navy on the basis of which contracts for the fractionation of human plasma were made with the seven commercial firms [18] stated:

E-2. Specifications for the preparation of human albumin from human blood plasma.

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setts General Hospital, Peter Bent Brigham Hospital; Chicago, Ill., Michael Reese Hospital; Iowa City, Iowa, University Hospital; New York, N. Y., Memorial Hospital, Presbyterian Hospital; Philadelphia, Pa., University Hospital; Washington, D. C., United States Naval Hospital, Walter Reed Hospital.

[18] It is a pleasure to acknowledge the collaboration of these firms: the Armour Laboratories, Cutter Laboratories, Lederle Laboratories, Inc., Eli Lilly and Company, Sharp & Dohme, Inc., E. R. Squibb & Sons, and the Upjohn Company.
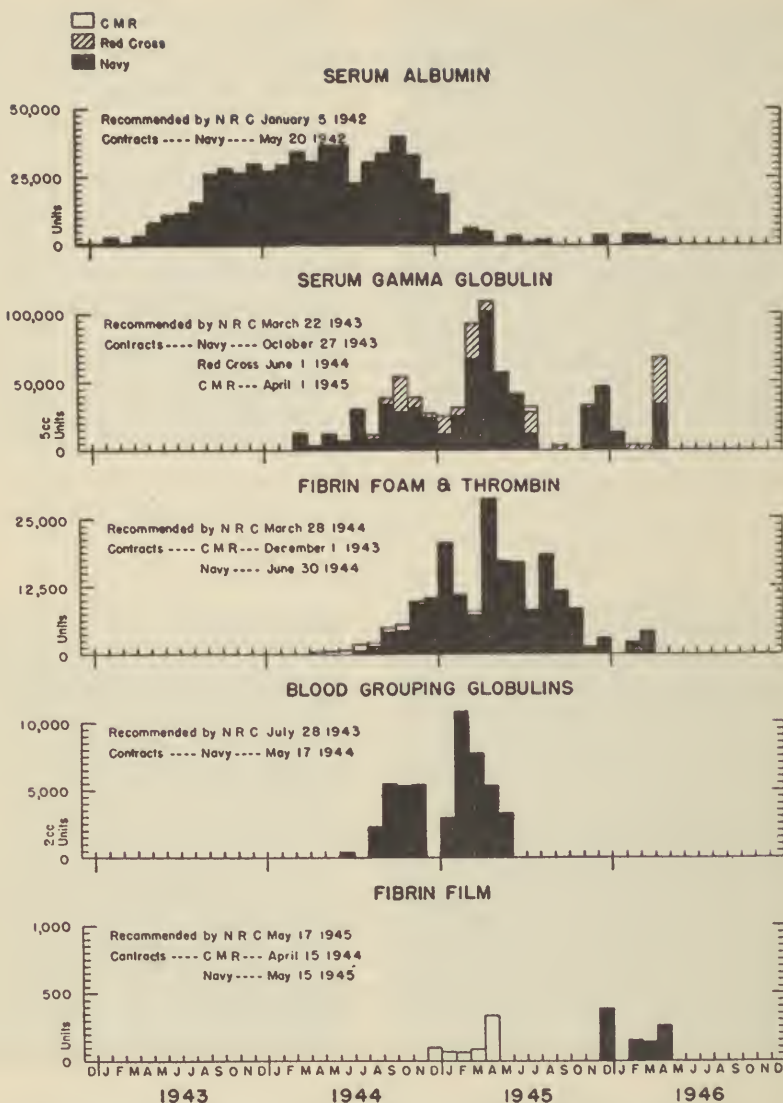
FIGURE 47. *Normal human plasma fractionation products.*

E-2a. Preparation of human plasma. — The plasma prepared from human blood shall meet minimum requirements of the National Institute of Health for Normal Serum Albumin (Human).

E-2b. Fractionation of human albumin from human plasma. — The process to be employed in the fractionation of human plasma, in order to yield albumin, is described in detail in the directions for the preparation of Normal Human Serum Albumin, which form a part of this specification. The entire process is carried out at temperature below zero centigrade. After the first step is completed at o degrees centigrade a minus 5 degrees centigrade cold room regulated to within 0.5 degree C. is necessary for the remainder of this process. The only reagents employed are ethanol-water mixtures, sodium acetate buffers and a filter aid (filter-cel or a similar substance). The ethanol-water mixture shall be construed to include the use of especially denatured 3A alcohol which is made by adding 5 gallons of methanol to each 100 gallons of ethanol. Only freshly distilled pyrogen free water and pyrogen free ethanol-water mixtures shall be used throughout this process. All equipment used must be chemically clean and free of pyrogenic substances. The process of fractionating human plasma at low temperature by ethanol-water-buffer-mixture divides plasma into six fractions.

E-2c. Fraction I. — This consists largely of fibrinogen. This fibrinogen fraction should be preserved at minus 5 degrees centigrade under 10 per cent ethanol containing 0.5 per cent sodium citrate for further purification. It should remain completely soluble and coagulable.

E-2d. Fractions II and III. — These represent both the gamma globulins of small and large molecular weights, and small amounts of beta globulin and prothrombin. It can be preserved for further purification at minus 5 degrees centigrade or retained in the frozen state.

The prothrombin remains active as tested in the manner described in "Appendix A." This fraction is also rich in antibodies and for this reason should be preserved by the commercial biological laboratory until released by the contracting authority.

E-2e. Fraction IV. — This is also rich in globulins, sterols and contains certain immune bodies. This fraction must be retained by the commercial biological firm until released by the proper authority.

E-2f. Fraction V. — This consists essentially of the albumin precipitated in an isoelectric condition. It is this fraction which yields the bulk of the albumin employed for transfusions.

E-2g. Fraction VI. — This represents the mother liquors. It contains small amounts of albumin as well as traces of globulins and presumably other proteins, fats and smaller molecules. It can be concentrated at low temperatures under reduced pressure in a glass lined or stainless steel still. The concentrate can be used for the recovery of albumin and the distillate for the recovery of alcohol.

E-2h. Specifications for the final product. — The solution shall contain 25% of human albumin processed from human blood by a method approved by the Plasma Fractionation Control Laboratory. The solution shall contain not more than 2% globulins. At the time of manufacture it shall be without significant turbidity or precipitate, as determined by visual inspection with the aid of a suitable light. It shall not undergo significant visible change when exposed to

50° C. for at least 12 days and following such exposure the injection of 100 cc. intravenously into man shall cause no untoward reaction. The approval and final release of the packaged albumin solution by the Plasma Fractionation Control Laboratory at the Department of Physical Chemistry, Harvard Medical School, Boston, Mass., acting for the U. S. Navy, shall constitute complete fulfillment of the conditions imposed by this paragraph.

E-2i. The final solution of albumin.

E-2i(1). Sterility tests. — Before being distributed the final product must be proven sterile by sterility tests as specified by the National Institute of Health.

E-2i(2). Addition of a preservative. — A preservative (bacteriostatic agent) shall be added according to the regulations of the National Institute of Health.

E-2i(3). Safety tests. — It must be proved nontoxic in guinea pig safety tests (5 cc. administered intraperitoneally in guinea pigs weighing 300 grams plus or minus 10%). These tests shall be carried out on two guinea pigs at the time the *final containers* are filled.

E-2i(4). Pyrogen testing of the final solution. — The contracting processor (biological laboratory) shall carry out pyrogen tests on rabbits in a manner similar to that outlined by the National Institute of Health in paragraph 12b. of Minimum Requirements for Normal Serum Albumin (Human). Dispensing of questionable lots of albumin shall be at the discretion of Dr. Edwin J. Cohn of the Harvard Medical School, Boston, Mass.

E-2i(5). Records of test. — Triplicate records of the bulk and final container sterility tests, animal safety and pyrogen tests shall be forwarded to the Clinical Testing Committee along with aliquots of the lot of albumin under test.

E-2i(6). Aliquots for Clinical Testing Committee. — The processor shall forward six 100 cc. bottles and six 10 cc. bottles from each lot of albumin to the Clinical Testing Committee. This committee will carry out further tests required by the Army and Navy. No lot of albumin can be distributed by the processor until the findings of the Clinical Testing Committee have been reported.

E-2j. Bulk sterility. — In order to prevent the use of an undue proportion of albumin from each lot for testing purposes, it is recommended that bulk testing be carried out on not less than 20 liter lots.

E-2k. Contaminated plasma. — If sterility tests on the pooled plasma show it to be contaminated, it shall be processed and a report made to the general supervisor of albumin preparation.

E-2l. Control of processing.

E-2l(1). The technicians who are to be in charge of the fractionation of albumin in the contracting laboratories shall be sent to the Harvard Plasma Fractionation Laboratory for such instruction as will be specified by Dr. Edwin J. Cohn or his designated representative. Under the terms of the contract between the processing laboratory and the Navy, Dr. Cohn is recognized as the consultant in all matters related to the processing method. No variation in the manufacturing process shall be employed without his approval and each laboratory shall be open to inspection by Dr. Cohn or his representative at all times during the usual working hours. In addition, Dr. Cohn will be available for consultation at his office by appointment.

E-2l(2). Aliquot parts of the final product (albumin) prepared by commercial firms shall be forwarded to the Harvard Plasma Fractionation Laboratory, Bos-

ton, Mass., for the control of the product. All reports as to the acceptability of the products shall be made in triplicate.

E-2l(3). Approval of any lot of albumin by the Harvard Plasma Fractionation Laboratory, Boston, Mass., shall constitute an approval under this paragraph of the methods and processes employed in the manufacture thereof.[19]

PRESERVATION AND DEVELOPMENT OF OTHER PLASMA COMPONENTS

The very large amounts of the various globulins and of fibrinogen that were being separated and stored, under the best conditions that could at the time be specified, rendered it desirable to request that these components of plasma also be developed, and on March 1, 1942, the contract between the Office of Scientific Research and Development and Harvard University was extended to include "chemical, clinical and immunological investigations of bovine albumin and human and bovine fibrinogen, prothrombin, and other serum globulins." At this time also it was suggested that other laboratories be invited to collaborate in the plasma fractionation program, under OSRD (CMR) contract, in order to achieve regional distribution. On July 9, 1940, Dr. J. W. Williams of the University of Wisconsin had written us: "I understand the laboratories of Harvard University are being put on a war footing, and if you have any things with which we can help, I would appreciate suggestions." This offer was renewed on March 6, 1942, when he again offered "a collaboration in the work of the two laboratories, yours large and ours small. It was my idea we could in this way obtain in more efficient manner information about the serum proteins which could be used by workers in antitoxin laboratories, in the preparation of materials for transfusions, blood substitutes, and so forth, to further the health of the nation."

On the basis of this suggestion we recommended that a contract be made with the University of Wisconsin so that the facilities there for electrophoretic and ultracentrifugal analyses of protein solutions could be used in conjunction with ours in the control of the products being developed and produced. A contract was also made at this time with Stanford University that made available the services of Dr. J. Murray Luck for the control of processing in California. Both Dr. Williams and Dr. Luck spent many months in the Harvard Laboratories during the spring and summer of 1942 and subsequently in order to learn our processes and to collaborate in the control and further development of products of plasma fractionation.

Two further contracts were recommended to the Committee on Medical Research to aid in the control of this expanding program. One was with the Massachusetts Institute of Technology for the development by Dr. Hans Mueller of a nephelometer especially designed to facilitate the testing of the thermal stability of serum albumin, which, unlike dry plasma, was being

[19] Bureau of Medicine and Surgery, U. S. Navy, Specifications for Normal Serum Albumin (Human) Concentrated, 8 February 1943 superseding 28 July 1942.

distributed in solution and had to resist the most adverse climatic conditions of a tank in Tobruk or a landing in the Aleutian Islands.

All the above contracts had to do with the physical and physicochemical control of plasma proteins. The analytical knowledge of the amino acid composition of the various separated and purified proteins was also deemed necessary in the interest both of control and of further purification. A contract was therefore made with Columbia University for the services of Dr. Erwin Brand. These OSRD(CMR) contracts for research and development in universities and the Navy contracts for the production in commercial laboratories thus rendered nation-wide the program formulated in the spring of 1940.

Serum albumin was recommended to the armed forces in January 1942; γ-globulin in March 1943; the isoagglutinins, concentrated as blood-grouping globulins, in July 1943; fibrin foam and thrombin in March 1944; the new salt-poor serum albumin, heated in the final container so as to prevent virus transmission, in March 1945; and fibrin film in May 1945. Each of these products, first prepared in the Harvard Laboratories, had been licensed [20] by the National Institute of Health, recommended to the armed forces by the National Research Council, and produced under Navy contract at the times, and in the amounts, represented in Figure 47.

This steady development of new products of plasma fractionation continues. Albumin and γ-globulin, fibrinogen and thrombin, and the isoagglutinins were well-known components of plasma. Our first task, therefore, in the interest of the most economical use of the blood contributed to the Red Cross, was to separate each of these components and render them available in the most effective form for military use. These various products are illustrated in Figure 46.

Natural products rarely exist in a state of maximum purity or maximum concentration. They are found in the blood, as in other tissues, in the presence of other natural products, many of them in small amounts, or, as is the case with prothrombin and hypertensinogen, in inactive physical states in which they are held as reserves. Natural function may demand the liberation of the active component in but a small, constant concentration. The greatest value of each active component under pathological conditions may often prove to be as a highly purified reagent, stable and concentrated. Albumin, as supplied to the armed forces, was five times, and the γ-globulin antibodies twenty-five times, as concentrated as in plasma.

When the first contracts were made by the Navy for human serum albumin early in 1942, knowledge of the best conditions for the preservation of the other fractions was incorporated into the Navy specifications in the inter-

[20] In each case specifications were drawn in collaboration with the Director, Dr. Elliott Robinson, and later with the Acting Director, Dr. Geoffrey Edsall, of the federally licensed Massachusetts Antitoxin and Vaccine Laboratory.

est of the preservation of those plasma constituents whose importance had been recognized on the basis of earlier research (14).[14] By the time the Navy contracts were made in June 1945, for salt-poor albumin, the specifications had been modified from those of 1942–1943 to read:

E-2b. Preparation of human albumin from human plasma. — The process to be employed in the fractionation of human plasma to yield Albumin, Normal (Human) Serum, concentrated salt-poor, shall be that designated by the Director of the Plasma Fractionation Control Laboratory, Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, and as it may be modified from time to time by the Director.

E-2c. The Fractions other than albumin resulting from the fractionation of human plasma by the process designated above shall be processed and stored under ordinary refrigeration by the contractor in the following manner for a period of two years or less as may be modified by the purchasing activity:

Fraction I

In a dry sterile form in individual bottles containing 0.2 gram or 12.0 or 24.0 grams as determined by contract unless separately contracted for to make Fibrin Foams or Fibrin Films.

Fraction II + III

As a bulk dry powder (Fraction II).

Fraction III-2

Purify, convert to Thrombin and bulk dry.

Fraction IV-3, 4

In a dry sterile form (15 grams per bottle)

Fractions III-0 and IV-1

To be discarded.

All fractions shall be given lot (control) numbers indicating their source.

E-2d. Specifications of the final product. — The final solution shall conform to the minimum requirements of the National Institute of Health. It shall contain, in a suitable diluent, 25% human albumin not less than 97% pure as measured by electrophoresis with a barbiturate buffer and processed from human blood by a method approved by the Director of the Plasma Fractionation Control Laboratory. Unless otherwise specified, the solution shall contain 0.04 M sodium acetyltryptophane and not more than 0.33 grams % sodium. At the time of manufacture it shall have an initial low turbidity and shall show no visible haze after 12 days heating at 50° C. The injection of 100 cc. intravenously into man shall cause no untoward reaction. The approval and final release of the packaged albumin solution by the Director of the Plasma Fractionation Control Laboratory at the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, acting for the U. S. Navy, shall constitute complete fulfillment of the conditions imposed by this paragraph.

TABLE II

Antibodies in Globulin Fractions from Human Blood Plasma *

Fraction	Antibody	Type of Antibody	Investigator	Institution	Concentration Compared to Plasma
II + III†	Anti-diphtheria	Antitoxin neutralizing	Edsall	Massachusetts Antitoxin and Vaccine Laboratory	10
	Anti-dysentery	Agglutinins	Mudd	University of Pennsylvania	2-10
	Anti-herpes simplex	Neutralizing	Stokes	Children's Hospital, Philadelphia	‡
	Anti-influenza (human PR8)	Hirst inhibition	Hirst	Rockefeller Institute	4-8
	Anti-influenza (human PR8)	Hirst inhibition	Eaton	California Department Public Health	4
	Anti-influenza (human PR8)	Hirst inhibition	Enders	Harvard Medical School	10-15
	Anti-influenza (human PR8)	Complement fixation	Enders	Harvard Medical School	10-15
	Anti-influenza (human PR8)	Neutralizing	Stokes	Children's Hospital, Philadelphia	10
	Anti-influenza (human PR8)	Neutralizing	Enders	Harvard Medical School	9
	Anti-influenza (swine)	Neutralizing	Stokes	Children's Hospital, Philadelphia	10
	Anti-influenza (swine)	Neutralizing	Shafer	Massachusetts Antitoxin and Vaccine Laboratory	4
	Anti-lymphocytic chorio-meningitis	Neutralizing	Stokes	Children's Hospital, Philadelphia	‡
	Anti-measles	Protective (human)	Stokes	Children's Hospital, Philadelphia	2-10
	Anti-mumps	Complement fixation	Enders	Harvard Medical School	64
	Anti-parapertussis	Agglutinins	Mudd	University of Pennsylvania	4-10
	Anti-pertussis	Agglutinins	Mudd	University of Pennsylvania	10
	Anti-pertussis	Agglutination	Enders	Harvard Medical School	4-10
	Anti-pertussis	Mouse protection	Bradford	University of Rochester	‡
	Anti-perfringens	Protective	Hall	University of Colorado	10
	Anti-poliomyelitis	Neutralizing	Kramer	Michigan Department of Health	16
	Anti-poliomyelitis	Neutralizing	Stokes	Children's Hospital, Philadelphia	10
	Anti-poliomyelitis	Rat and mice protection	Kramer	Michigan Department of Health	10
	Anti-poliomyelitis	Rat and mice protection	Stokes	Children's Hospital, Philadelphia	‡
	Anti-scarlatina	Neutralizing	Bradford	University of Rochester	5-10
	Anti-scarlatina	Neutralizing	Wadsworth	New York Department of Health	4-10
	Anti-streptococcus	Antitoxin	Wadsworth	New York Department of Health	8-10
	Anti-typhoid	H agglutinin	Enders	Harvard Medical School	

	Anti-typhoid	O agglutinin	Enders	Harvard Medical School	8-10
	Anti-vaccinia	Neutralizing	Janeway	Children's Hospital, Boston	‡
	Isoagglutinins	Agglutinins	Boyd	Harvard Medical School	8-10
I	Anti-typhoid	O agglutinin	Enders	Harvard Medical School	< 1
	Anti-typhoid	H agglutinin	Enders	Harvard Medical School	1
	Anti-mumps	Complement fixation	Enders	Harvard Medical School	< 1
	Anti-influenza (human PR8)	Complement fixation	Enders	Harvard Medical School	< 1
	Anti-influenza (human PR8)	Neutralizing	Enders	Harvard Medical School	< 1
	Anti-diphtheria	Antitoxin	Enders	Harvard Medical School	< 1
IV	Anti-typhoid	O agglutinin	Enders	Harvard Medical School	2
	Anti-typhoid	H agglutinin	Enders	Harvard Medical School	< 1
	Anti-influenza (human PR8)	Complement fixation	Enders	Harvard Medical School	< 1
	Anti-diphtheria	Antitoxin	Edsall	Massachusetts Antitoxin and Vaccine Laboratory	1
	Anti-dysentery	Agglutinins	Mudd	University of Pennsylvania	1
	Isoagglutinins	Agglutinins	Boyd	Harvard Medical School	< 1
Supernatant of II + III	Anti-typhoid	O agglutinin	Enders	Harvard Medical School	< 1
	Anti-typhoid	H agglutinin	Enders	Harvard Medical School	1 or < 1
	Anti-mumps	Complement fixation	Enders	Harvard Medical School	< 1
	Anti-influenza (human PR8)	Complement fixation	Enders	Harvard Medical School	< 1
	Anti-influenza (human PR8)	Neutralizing	Enders	Harvard Medical School	< 1
	Anti-diphtheria	Antitoxin	Enders	Harvard Medical School	< 1

* Enders, J. F., *J. Clin. Invest.*, 23, 510 (1944). Table taken from pages 515–516.

† These assays were undertaken at the request of Dr. A. R. Dochez of the Committee on Medical Research of the Office of Scientific Research and Development, who wrote to the investigators listed in March 1942: "In the process of preparing human plasma used for transfusion purposes in the armed forces of the United States, by Dr. Edwin Cohn of Harvard University, a number of fractions of the original plasma result. Only one of these, the albumin fraction, is used for transfusion. It is the desire of the Government to ascertain to what useful purpose the remaining fractions can be put. Among these fractions is one containing the α-, β-, and γ-globulins. As you doubtless know, this fraction contains whatever immune bodies may have been present in the original plasma. In the process of purification approximately ten times concentration of the immune body fraction is effected. It is hoped that these immune bodies may be used practically either for the prophylaxis or treatment of certain infectious diseases. In order to test the validity of such a procedure it is first necessary to titrate the globulin fraction for its content of specific antibodies. . . . The first titrations would be with mixtures of the α-, β-, and γ-globulins. Later fractionation of the different globulins will be performed and the specific immune body containing globulin will be furnished for a similar titration." We are greatly indebted to Dr. W. C. Boyd for compiling this table.

‡ Activity present but no quantitative data.

Comparison of these specifications with the earlier ones (pages 375–379) reflects the research and development that had taken place under OSRD (CMR) contract. Salt-poor albumin was distributed without a mercurial preservative. It was rendered thermostable by the addition of nonpolar anions (17) and heated in the final containers for ten hours at 60° C. (18) to prevent virus transmission (19). Fraction I was either processed directly to fibrin foams or films or dried to preserve fibrinogen or the antihemophilic globulin. Indeed, all fractions, save III–o and IV–1, in which the lipoproteins were concentrated, were now dried so as to render stable as many as possible of their valuable known and undiscovered components. Research regarding the nature of the latter as well as of the lipoproteins continued.

Although the methods were "classified," it seemed wise to arrange for the publication in one place of the characterization of the various products that had been developed and the uses being found for them. A series of papers on the chemical, clinical, and immunological studies on the products of human plasma fractionation therefore appeared in the July 1944 number of the *Journal of Clinical Investigation* and subsequent issues (20–47).[21]

The need for greater knowledge, not only about the major but also the trace components of blood, led us continuously, since the early days of the program, to make available to interested and competent investigators small amounts of the fractions being separated in order to explore, by analytical or other means, in which fraction each component was concentrated. This survey was organized in the case of the antibodies by Dr. A. R. Dochez of the Committee on Medical Research, by Dr. W. C. Boyd of the Boston University School of Medicine, collaborating and consulting under our contract from November 1, 1941, until the end of the war, and by Dr. J. F. Enders of the Harvard Medical School, collaborating under our contract since March 1, 1942, when it was extended to include antibodies. The results of this survey are given in Table II.

These early studies demonstrated that essentially all antibodies were concentrated in Fraction II + III. The further concentration achieved by the separation of human antibodies and of animal antitoxins into Fraction II and Fraction III–1 has led to the preparations now available, in which the concentration is twenty to twenty-five fold that in plasma. Still greater concentration may be anticipated if specific antibodies are separated from each other.

A survey for other substances of chemical, clinical, and immunological significance was begun in 1941 by Dr. Hubert Vickery of the Connecticut Agricultural Experiment Station, who worked in the Department of Physical Chemistry at the Harvard Medical School under our contract after the Committee on Medical Research had been founded, carrying forward this study

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[21] References to several papers (2, 17, and 18), also a part of this series, have already been made.

and aiding in the control of commercial production in the period of its expansion, 1942 to 1944. The knowledge, born of this attempt to determine the distribution and concentration of the components of plasma, summarized in Table IX, accumulated as the major components of value in military medicine were separated and concentrated into fractions by increasingly refined procedures.

II. PREPARATION OF PLASMA FRACTIONS

The system that has been developed and employed for the separation into fractions of the protein and lipoprotein components of the plasma is an outgrowth of theoretical physicochemical studies and is applicable to other biological tissues and fluids. The highly specific forces, on which the interactions of proteins and low concentration of electrolytes depend, are masked in the concentrated salt solutions that have heretofore generally been used for protein precipitation and purification. Proteins are, however, precipitated by water-miscible organic liquids. At ordinary temperatures such organic liquids "generally lead to protein denaturation. Irreversible changes in the labile protein molecules can generally be minimized, however, if the temperature is maintained sufficiently low. Thus egg albumin, a readily denatured protein, could be recrystallized after being in 25 per cent ethanol at $-5°$ for a month, if the ethanol was removed before the temperature was raised (48). Neutral salts increase solubility under these conditions much as they do the solubility of globulins in water, and this observation has been extended to other proteins (49)."

Reducing the solubility of a protein to any desired extent by the addition of a water miscible organic liquid, at a temperature sufficiently low to prevent protein denaturation, has both theoretical and practical advantages. From a practical point of view the volatile organic liquid can be removed at low temperature. A most convenient procedure for this operation consists in lowering the temperature sufficiently below that employed in processing to freeze the wet protein precipitate, and then removing both the organic liquid and the water under reduced pressure (50).* The dangers of bacterial growth which beset dialysis are completely avoided, and the final product is a dry preparation of the protein and of such non-volatile substances as precipitate with it. . . .

In the fractionation of plasma proteins that has been carried out for the Armed Forces on a large scale with the blood of over two million donors to the American Red Cross, a five-variable system has sufficed in which the limits were as follows:

* The process of drying proteins from the frozen state yields soluble, undenatured preparations in most cases. For certain lipoproteins, however, freezing appears to weaken the attachment between lipid and protein. This effect is even greater if the freezing takes place in the presence of an organic liquid. Indeed, freezing in the presence of a lipid solvent insoluble in water has been used as a method to remove lipid from the protein to which it is attached. The removal of organic precipitants from lipoproteins thus raises special problems.

Variable	Limits Employed		
pH	4.4	to	7.4
$\Gamma/2$	0.001	to	0.16
Ethanol concentration, mole fraction	0	to	0.163
Ethanol concentration, vol. % at 25°	0	to	40
Protein concentration, g./l.	0.2	to	66
Temperature, ° C.	0	to	−10

These conditions have been attained, and the variables maintained constant, by the use of acetate and carbonate buffer systems to control pH and ionic strength, and by the use of ethanol as precipitant since it was readily removed by distillation. These reagents are both convenient for the processing of biological systems and safe to use in the preparation of protein and lipid products, even where large quantities are destined for intravenous use. (51, pp. 461–462.[1])

The procedural details for carrying into large-scale production the separations determined upon on the basis of theory and experiment were devised in the Harvard Pilot Plant largely by Dr. L. E. Strong and his co-workers. In practice,[2] the plasma separated from the centrifuged blood was brought to 0° C. Ethanol and buffer were then added through capillary tubes at a slow rate, so as to avoid local excess of reagents, and the temperature was lowered so as to maintain the solution close to the freezing point until the ethanol was 8 volume per cent,[3] the temperature − 3° C., the pH 7.2, and the ionic strength 0.14.

Fraction I

After equilibration the precipitate was removed by centrifugation; in large-scale production in the Sharples centrifuge (Fig. 45). This precipitate, designated Fraction I, contained most of the fibrinogen and the anti-hemophilic globulin. It was further purified by reprecipitation, under specified conditions, by sterile filtration and preserved by drying from the frozen state.[4]

Fraction II + III

Following the removal of Fraction I, the ethanol concentration of the solution was slowly and carefully increased, as before, to 25 volume per cent, the temperature being simultaneously decreased to −5° C., the pH to 6.8, and the ionic strength to 0.09. The resulting precipitate, Fraction

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[1] Footnotes and bibliographical reference numbers in this quotation have been changed to make them conform to those in this chapter.

[2] Practice is here illustrated by the most recent procedures recommended, which were specified in the Navy contracts of 1945; Method 6 for plasma fractionation (51) and Method 9 for II + III subfractionation (52).

[3] Values of volume per cent and pH refer to the same systems at 25° C.

[4] See Section VIII.

II + III, contained all the antibodies, the isoagglutinins, prothrombin, and the β-lipoproteins.

In earlier procedures, Fractions II and III were precipitated separately (13).[5] Since prothrombin and the γ-globulins were found in both these fractions, however (see Table I), they were later precipitated together and then each purified by subfractionation. In the procedure now in use the lipoproteins were first separated. This was accomplished by reprecipitation of an almost lipid-free Fraction II + III–w in 20 per cent ethanol at $-5°$ C., pH 7.2, and ionic strength 0.005. Such low ionic strengths are readily reached in our process, by reprecipitation from salt-poor alcohol-water mixtures, without recourse to dialysis. The solution contains essentially all the β-lipoproteins. They have been reprecipitated by adjusting the pH to 5.7, the ethanol concentration to 25 per cent, and the temperature to $-5°$ C. The separated fraction, which contains the so-called X-protein (53) of plasma, is designated as III–o. The lipoproteins, containing as much as 75 per cent lipid, that have been separated from Fraction III–o by euglobulin precipitation and ultracentrifugal purification (52) are among the most interesting blood components being made available for further investigation. They are carriers of steroids such as estriol and vitamin A.

Following this reprecipitation Fraction II + III–w, nearly lipid-free, could be dried from the frozen state. It still, however, contained essentially all antibodies,[6] as well as isoagglutinins and prothrombin and plasminogen, the precursor of the fibrinolytic enzyme. It was resuspended in an acetate buffer, the pH was adjusted to 5.2 the ionic strength to 0.015, and the ethanol to 17 per cent at $-6°$ C.[7] The precipitate, Fraction III, contained the isoag-

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[5] See footnote 14, Section 1.

[6] The γ-globulin antibodies that were concentrated in Fraction II, in the best method (Method 3) available at the time the first contracts were made, were neither as pure, as stable, nor as high in yield as those achieved by the present process. Work during 1943 and 1944 led to a series of modifications of Method 3 that had as their goal the production of γ-globulin of higher purity in the hope that it could be injected intravenously. Methods 3A and 3C were widely used in commercially produced materials, and a large number of experimental products were prepared at the Harvard Pilot Plant for study by Dr. Janeway and his associates. Methods 4–7 were attempts to improve the isolation of the isoagglutinin fraction. In the fall of 1944 new studies were undertaken, both at Harvard and Wisconsin, with the aim of increasing the yield of γ-globulin and isolating a β-globulin fraction in a more nearly "native" state. At the same time, it was necessary to prepare both prothrombin and isoagglutinin fractions. Work at Wisconsin led to a simplified procedure (Method 4W) producing a better yield of γ-globulin in Fraction II by employing an ionic strength lower than had been used in previous commercial fractionation procedures (54). The Harvard group developed a procedure (Method 8) for the separation of the lipoprotein fraction (III–o) consisting largely of β-globulin, in a state much like that found in plasma, and of fractions containing prothrombin (III–2), isoagglutinins (III–1), and γ-globulin (II). It became apparent that these methods could be combined to considerable advantage, and Method 9 was the result of this combination.

[7] These are the conditions of pH, temperature, and ethanol concentration employed in globulin Method 3F with the lower ionic strength recommended by Deutsch, Alberty, and Williams (54) and incorporated in Method 9 (52).

glutinins, prothrombin and plasminogen. The protein in the supernatant was precipitated by readjusting the pH to 7.4 and the ethanol concentration to 25 per cent at $-5°$ C. to give a total Fraction II, which contained the antibodies that have proved to be of value in measles. The effectiveness of γ-globulin in measles and infectious hepatitis was, however, demonstrated with Fraction II prepared by Method 3. Fraction II prepared by Method 9 was therefore separated into two fractions, the more soluble of which, Fraction II–1, 2, consisted of γ-globulins soluble in 17 per cent ethanol at $-6°$ C., pH 5.2, and ionic strength 0.06; the remainder, Fraction II–3, has now also been demonstrated to be satisfactory for measles prophylaxis but not as yet for infectious hepatitis. The accompanying table gives the relative concentrations of the various antibodies in these fractions.[8] The results suggest that the antibodies in Fraction II are also subject to further subfractionation. Clinical comparison of the potency for measles prophylaxis of Fraction II–3 with that of Fraction II–1, 2 also suggests a ratio of approximately 0.7, as in the case of diphtheria antitoxin. Since Fraction II–3 only constitutes 30 per cent of the γ-globulin and is so close to II–1, 2 in potency, the two fractions have been combined in the preparation of γ-globulin for measles prophylaxis.

Antibody	Ratio of Concentration of Antibody in Fraction II–3 to That in Fraction II–1, 2
Diphtheria antitoxin	0.7
Streptococcal antitoxin	1.0
Influenza A, Hirst test	1.0
Influenza A, mouse protection	1.3
Typhoid H agglutinin	1.0
Typhoid O agglutinin	3.0

The precipitate, Fraction III, was resuspended at pH 5.4 at an ionic strength of 0.08 in water at the freezing point. The globulin precipitate (Method 9) (52), separated under these conditions, closely similar to those originally recommended by Mellanby (55), contained prothrombin, traces of fibrinogen, and plasminogen. The separation of prothrombin from plasminogen and the further purification and conversion of each to an active state need not be considered here (56).[9] The thrombin in Fraction III–2 (32) has been made available to the armed forces in large amounts for use as a hemostatic agent in connection with fibrin foam (37, 38, 57, 58).

The solution from which the prothrombin and plasminogen had been precipitated still contained the isoagglutinins as well as certain antibody eu-

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[8] As determined by Dr. J. F. Enders and Miss J. C. Sullivan in their systematic studies of the antibody contents of γ-globulin preparations being accepted for use by the armed forces and the American Red Cross (27).

[9] See also Section VIII.

globulins, including the typhoid O agglutinin. This fraction, III–1, may be of great importance in the case of other antibodies also; especially for such animal antitoxins as are in horse anti-plague serum.[10] Methods for the direct precipitation of the euglobulins in Fraction II + III–w were also found useful (Methods 6 and 7) (52). They were readily precipitated near pH 6.3, at an ionic strength depending on the alcohol concentration and temperature. Further purifications are therefore possible depending on the isoelectric points of the euglobulins and the range of ionic strengths in which they are respectively precipitated and dissolved.

Fraction IV

All the globulins remaining in solution after the precipitation of Fraction II + III at pH 6.8 and 25 per cent ethanol at − 5° C. precipitated when the pH was adjusted with acetate buffers to 5.8[11] and the ethanol to 40 per cent at constant temperature. The lipoproteins precipitated under these conditions were, however, denatured. In the process that has been gradually developed and that is now in general use, the α-lipoproteins in Fraction IV as well as the β-lipoproteins in Fraction III–0 are separated under far more satisfactory conditions. Indeed, all fractions may now be made available in a soluble state for further chemical, physiological, and clinical investigations.

The lipid-rich α-globulins were removed as Fraction IV–1 (Method 6) by decreasing the pH to 5.2 and the ethanol content to 18 per cent at − 5° C. Fraction IV–1 can be readily separated into three subfractions.

One of these fractions contains most of the lipid of the plasma that is combined with α-globulin, has a low solubility in water near pH 5.2, is soluble at pH 5.8 and 4.8, and is readily precipitated at the latter reaction by even low concentrations of neutral salt.* Another component contains the blue-green pigment previously described in one or another study of plasma proteins (64, 65), and appears to be isoelectric at an even more acid reaction. . . .

The precipitation at pH 4.7 and [25 per cent] ethanol at − 5° of all the proteins remaining in solution after the precipitation of Fraction IV–1 would have definite advantages, once satisfactory procedures were developed for the separa-

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* This "effect of minute quantities of acid on the solubility of a globulin in salt solutions" was early observed by Osborne and Campbell (59) and has since been repeatedly studied (60–63).

[10] Studies of the concentration of the antibodies in the anti-plague serum prepared by Dr. Karl F. Meyer under contract with the Office of Scientific Research and Development have been carried out in collaboration with Dr. E. E. Baker of the George Williams Hooper Foundation, Drs. J. D. Porsche and J. B. Lesh of the Armour Laboratories, and Drs. L. E. Strong and N. Mittelman in this laboratory. This material is insoluble in water at 0° C., at pH 6.3, at an ionic strength of 0.02 but completely soluble at this pH and temperature at an ionic strength of 0.15.

[11] In the earlier methods the pH was 5.5 (13). See also footnote 66, page 470, ref. 51.

tion by subfractionation of the lipid-poor α- and β-globulins (IV–4) † and the albumins (V). The molecular dimensions and osmotic behavior of the globulins in Fraction IV–4 resemble those of the albumins in Fraction V rather than those of the other globulins of the plasma (66). Fraction IV–4 precipitated in combination with Fraction V under these conditions contained nearly all of the hypertensinogen ‡ of plasma, whereas precipitation of Fraction IV–4 by [40 per cent] ethanol at pH 5.8 and −5° led to destruction of hypertensinogen and presumably of other labile components of the fraction without, however, destroying gross molecular structure. Fraction IV–4 contains the esterase of plasma § and an iron-binding globulin fraction.|| [12] (51, pages 469–470 [13])

    † This fraction was designated IV–3, 4 in previous communications.
    ‡ We are indebted to Dr. Lewis Dexter in the Department of Medicine of the Harvard Medical School for the assay of hypertensinogen.
    § We are indebted to Dr. Ralph W. Brauer in the Department of Pharmacology of the Harvard Medical School for the assay of esterase.
    || Personal communication from A. L. Schade, in press in *Science*.

Subfractionation of Fraction IV–4 has recently been achieved. Essentially all the lipoprotein has been precipitated in Fraction IV–5 from water at 0° C. at pH 4.9 at an ionic strength of 0.005. At 18 per cent ethanol and − 5° C. at pH 4.8–5.2 serum esterase was insoluble at ionic strength 0.02 and was separated and further purified from Fraction IV–6. The metal-binding protein remained in solution, from which it was precipitated, as Fraction IV–7, by returning to the original conditions, pH 5.8 and 40 per cent ethanol, for separating these globulins from albumin.

Fraction V

Fraction V, which contains most of the albumin, has been precipitated near its isoelectric point and purified by essentially the same procedures throughout this work (14, 51). Albumin has been crystallized from ethanol-water mixtures, but for clinical use there has been no advantage in achieving this additional purification, and the standard human serum albumin that has been made available to the armed forces in such large amounts has not been crystallized.

The distribution into fractions of these various components of plasma achieved by this process is given in Table III and is graphically represented in Figure 48, which indicates accurately the proportions of the proteins separated in each fraction and suggests also the uses that have thus far been found for each.

    [12] Footnotes and bibliographical reference numbers in this quotation have been changed to make them conform to those in this chapter.
    [13] Since this chapter was written the influence of plasma in retarding the growth of certain bacteria requiring iron for their metabolism, noted by Schade (169), has led to the further concentration of the plasma protein responsible for binding not only iron but copper and probably zinc (page 372, above); to its characterization as a β_1-globulin; and to its crystallization in collaboration with B. A. Koechlin on February 10, 1947.

Table III *

Distribution of Plasma Proteins into Fractions

Fraction	Albumin	α	β	γ	Fibrinogen	Total
		(Grams per liter of plasma)				
I	0.2	0.3	0.5	0.3	2.1	3.4
II + III	0.8	1.1	9.1	7.0	1.0	19.0
IV–1	0	4.5	0.5	0.1	0	5.1
IV–4	0.9	2.7	2.2	0	0	5.8
V	29.9	1.3	0.3	0	0	31.5
VI	0.8	0.2	<.1	0	0	1.0
Totals	32.6	10.1	12.6	7.4	3.1	65.8
Plasma	36.3	9.2	10.6	7.2	2.5	65.8

* Taken from Table IX, Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L., *J. Am. Chem. Soc.*, 68, 459, 1946.
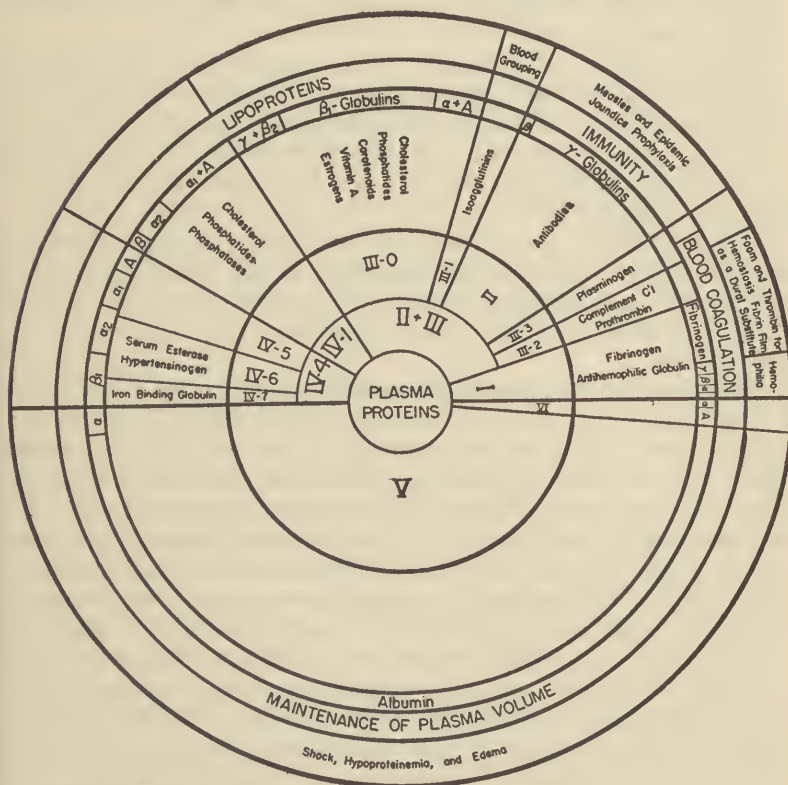
FIGURE 48. *Plasma proteins: their natural functions and clinical uses and separation into fractions.*

III. CRYSTALLIZATION OF HUMAN AND BOVINE
SERUM ALBUMIN

CRYSTALLIZATION OF SERUM ALBUMINS

Crystals of bovine serum albumin were first observed on June 20, 1941. They were long and needlelike and separated from 15 per cent ethanol. Since crystallization permitted the preparation of albumin of great purity, efforts were immediately directed, in collaboration with Dr. W. L. Hughes, Jr. (67), toward perfecting crystallization procedures. In the course of the next few months Dr. Heyl and Dr. Janeway were able to show, first by skin tests and subsequently by intravenous injection, that bovine serum albumin, purified by repeated crystallization, was freed from substances causing immediate reactions.

While attempting to determine better conditions for crystallization, Hughes found in August 1941 that albumin crystallized readily and in good yield from 40 per cent ethanol.[1] Albumin, crystallized one or more times from 40 per cent ethanol, was readily and reproducibly recrystallized from 15 per cent ethanol to give the first type of crystals discovered. Both types are illustrated in Figure 49.

Studies on content of carbohydrate and globulin impurities indicated that crystallization of the needle type separating at low alcohol concentration achieved additional purification. Although the yield under these conditions was poor, it seemed probable that a true fractionation of the serum albumins was obtained.

Human serum albumin was first crystallized from 40 per cent ethanol in collaboration with Mr. J. H. Weare. The crystals separated from albumin that had been concentrated in a vacuum still to which decanol had been added to prevent foaming. Attempts to crystallize, under these conditions, albumin precipitated as Fraction V, rather than concentrated in the still, were unsuccessful, until in June 1942 Hughes added decanol to the system, and the albumin readily crystallized. Further research revealed that other aliphatic alcohols containing six or more carbon atoms were effective in facilitating crystallization, as were toluene and benzene. Use of ethyl ether in conjunction with decanol reduced several fold the amount of alcohol necessary for crystallization. Such preparations, especially since crystallization was possible at lower alcohol concentrations, had very great thermal stability. Thus, a preparation (HA 64) twice crystallized from 25 per cent ethanol with a minimum amount of decanol and ether and with the temperature

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[1] These crystals appeared as well-formed hexagonal prisms with truncated ends provided the ionic strength was greater than 0.4. At lower ionic strengths the crystal angles were poorly defined. The crystals showed a marked temperature coefficient of solubility.

maintained as low as possible proved the most stable human serum albumin thus far obtained and has been extensively studied (23). Human serum albumin recrystallized with these aids proved satisfactory in the clinic.

CRYSTALLIZED BOVINE SERUM ALBUMIN AS A BLOOD SUBSTITUTE [2]

Histological, immunological, and clinical studies (16, 68, 69) on crystallized bovine serum albumin were being carried on throughout the period (1941–1945) during which these chemical studies were in progress. The first small-scale preparations (CB 1–2) were made available for clinical study during November 1941. Larger amounts were then crystallized in the Harvard Pilot Plant (CB 3–7) from Fraction V from bovine plasma fractionated at the Armour Laboratories. In April 1942, at the time the Harvard Pilot Plant was given over to instructing representatives of industrial firms with Navy contracts in the process of fractionating human plasma, Drs. J. D. Porsche and J. B. Lesh of the Armour Laboratories, after considerable experience at the Harvard Pilot Plant, returned to Chicago with a memorandum [3] giving procedural details and suggestions for improvements for future preparations of crystallized bovine serum albumin. Larger preparations (ACB 1–5) were soon forthcoming.[4] Clinical results with early preparations had been so encouraging that the testing program was expanded to include, besides Dr. Janeway's group, groups under Dr. Seegal in New York, Dr. Blalock in Baltimore, and Dr. Wangensteen in Minnesota, and every effort was made to increase the production of crystallized bovine serum albumin as rapidly as possible.

However, in September 1942 the use of bovine serum albumin appeared contraindicated by the results of extended clinical trials, for a number of serious cases of delayed serum sickness, of a kind not previously observed, were reported. The chemistry of the preparations was reviewed, and the only difference noted between these and the earlier, apparently successful, preparations was in thermal stability. It was thought possible that denatured albumin, indicated by low stability, being much less soluble, might be more antigenic. Conditions were determined for the crystallization of albumin of very high stability.[5] However, since injection of very stable crystallized bovine serum albumin (CB 25) led to a case of serum sickness, it was de-

[2]. Clinical studies on crystallized bovine serum albumin are further considered in Chapter XXIX.

[3] This memorandum, prepared at the request of the National Research Council, was submitted for transmission to Great Britain on April 11, 1942.

[4] Prepared at the Armour Laboratories under token contract with the Office of Scientific Research and Development.

[5] The revision of the procedures for the crystallization of albumin that led to preparations CB 21–26 and subsequent preparations were described in a memorandum first submitted November 4, 1942, and revised December 15, 1942.

cided that instability could not be the only factor in the incidence of serum disease. It was next suggested by Dr. C. v. Z. Hawn that the mercurial, merthiolate, that had been added to all but our early preparations, might have enhanced the antigenicity of the albumin. Clinical results were encouraging [6] with the next preparation (CB 26) distributed without a mercurial, but later preparations (ACB 29–32), although carefully analyzed for traces of metals, also produced delayed serum sickness in about one in ten persons receiving large amounts.[7] The possibility that the most insoluble fractions being prepared and used in clinical trial might be more antigenic than more soluble fractions led to the trial of crystals separated from mother liquors. The first of these preparations (ACB 33) was given to 14 persons without untoward results of any kind. The next (ACB 41), given to 57 persons, resulted in 2 delayed reactions. The next two preparations (ACB 44 and 47), given to 39 persons, resulted in 7 cases of delayed serum sickness. Consequently at this time it was decided that the production of a bovine serum albumin safe for intravenous use must await some future discovery, and clinical testing was temporarily discontinued in April 1945.

INTERACTIONS OF SERUM ALBUMIN AND MERCURY

The use of mercurials, as preservatives, for serum albumin,[8] as for plasma, was specified by the National Institute of Health. It was early observed by Lieutenants Woodruff and Gibson that if mercurials were added before fractionation mercury was found concentrated in the albumin fraction. Chemical observations on this interaction have followed recent attempts [9] to remove the mercury from substandard, dry plasma in order that it might be salvaged by fractionation to yield fibrinogen and thrombin, γ-globulin, and albumin processed to the salt-poor state and distributed without a mercurial according to the latest specifications.[10]

It was found possible by Dr. Hughes to remove the approximately 1 mg. per cent of mercury present as an organic mercurial in the plasma by adding 0.002 M of cysteine to the solution before precipitation of the albumin. This

[6] Clinical testing, which had been interrupted in September 1942, was resumed by Dr. Wangensteen when the new preparations (CB 25–26 and ACB 29–47) became available.

[7] At this time (February 21, 1944), a "Report on the Relative Merits of Blood Derivatives and of Various Suggested Blood Substitutes" was prepared "for study by members of Senate Military Affairs Committee charged with considering full mobilization of technological resources for war effort" and "authorized by the Office of Scientific Research and Development to comply with request from Senator H. M. Kilgore for a report of research under Contract OEM cmr-139."

[8] See page 378, E–2i(2).

[9] This attempt, made at the request of the American Red Cross, was carried out in collaboration with the Squibb Laboratories.

[10] See page 381, E–2d.

procedure was suggested by the known affinity for mercury and other metals of thiols such as cysteine and related molecules including British anti-lewisite (BAL).[11]

In the course of this investigation it seemed desirable to determine the number and the nature of the reactive groups of the albumin that were in combination with the mercurial. When small amounts of mercuric chloride were added to an albumin solution, crystals of a new type appeared. These crystals, illustrated in Figure 49, are being further studied.[12]

INTERACTIONS OF SERUM ALBUMIN AND SMALL ORGANIC MOLECULES

Human serum albumin, crystallized from ammonium sulfate, had previously been reported to contain considerable amounts of fatty acids (71). Albumin, both of human and bovine plasma, crystallized from ethanol-water mixtures, has also been demonstrated to contain fatty acids, although in smaller amounts of approximately 0.3 per cent. These fatty acids have recently been removed without sensibly damaging the albumin by extracting the dried powder at low temperature. Bovine serum albumin was then re-crystallized from 40 per cent ethanol, giving crystals similar to those previously observed. Crystallization from lower ethanol-water mixtures has, however, been achieved only when the extracted substances, presumably fatty acids, were again added. The interaction of albumin and fatty acids has been investigated by observations on stability and electrophoretic mobility (17, 72) and on bacterial growth (73), as well as on solubility and crystallization. Albumins also interact with simple salts (74), with alkyl sulfates (75), and such water-insoluble organic molecules as naphthoquinones,[13] with such organic metal complexes as mercurials, and with sulfonamide drugs (76). The nature of the protein groups involved, the lability of the complexes, and their possible role, on the one hand in transport and on the other in antigenicity, must be further investigated.

IV. CHARACTERIZATION OF PLASMA PROTEINS

ELECTROPHORESIS

The electrophoretic apparatus of Tiselius (77) has proved extraordinarily useful in the control of plasma fractionation by reason of both ease and re-

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[11] See pages 543, 555–561, and Hellerman (70).
[12] Since this chapter was written these crystals have been shown by Dr. Hughes to contain one mole of mercury combined with two moles of albumin. Neutral solutions of these crystals have also been demonstrated, in the ultracentrifuge, to contain albumin of double molecular weight in equilibrium with albumin of normal size into which the dimer molecules decompose.
[13] Personal communication from H. Heymann and L. F. Fieser, *J. Am. Chem. Soc.*, in press.
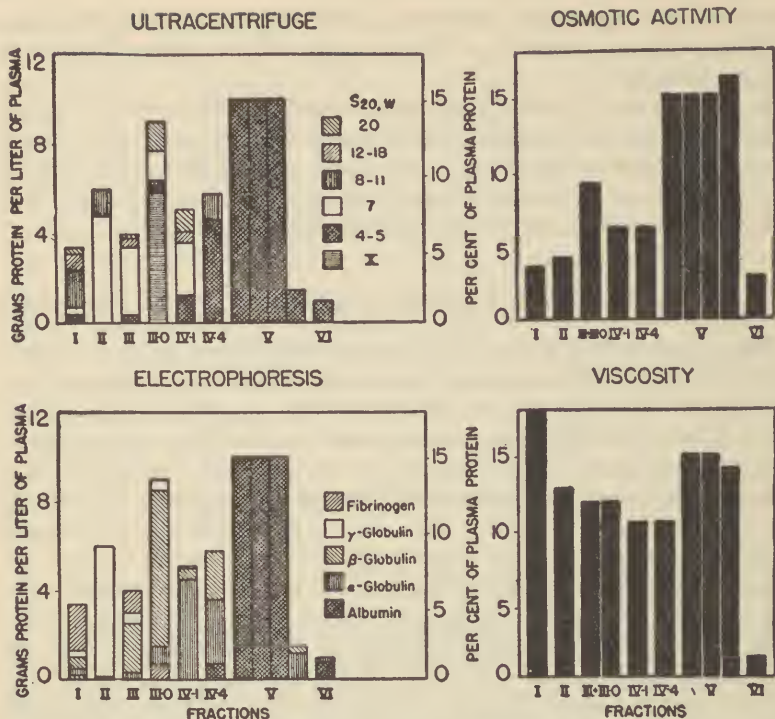
The distribution of components of various sedimentation constants in normal human plasma is represented in the upper left graph. The distribution of components of various electrophoretic mobility is represented in the lower left graph. The contribution to the osmotic activity has been estimated (upper right graph) as x/c, in which x is the weight of the fraction in one gram of plasma protein, and c is the concentration of the fraction which exerts the same osmotic pressure as plasma. The contribution to the viscosity has been estimated (lower right graph) as x/c' in which c' is the concentration of the fraction which has the same viscosity as plasma. In certain cases the bars are drawn side-by-side rather than as a single bar, in order to keep them on the same scale as the other fractions.

FIGURE 50. *Characterization of human plasma proteins.*

producibility of analyses. Motion in an electric field depends on many factors,[1] the most important of which is the ratio of the electrical charge to the surface of the protein molecule. It therefore varies with the pH, the ionic strength, and the nature of the buffer used.[2] Although this method sometimes groups together components with diverse sizes, shapes, chemical com-

[1] Mueller, H., Chapter 25 on "The Theory of Electrophoretic Migration," in *Proteins, Amino Acids and Peptides,* by E. J. Cohn and J. T. Edsall, Reinhold Publishing Corp., New York, 1943.
[2] Although much of the earlier work was done with a phosphate buffer of pH 7.7, we later adopted the diethylbarbiturate buffer of pH 8.6 (78).

positions, or solubilities, the resultant quantitative characterization of plasma proteins, under standardized conditions, has sufficient correlation with both physicochemical and biological properties to render the electrophoretic characterization of each fraction most useful from the standpoint of the development (13, 14, 20, 51) and of the control of uniformity of the fractionation process and in the estimation of purity (21) and yield (79) of the various fractions.

The distribution of the electrophoretically distinguishable components in the various fractions is graphically represented in Figure 50. The albumin in plasma moves with the greatest mobility because of its acid isoelectric point, its high net charge at neutral and slightly alkaline reactions, and its relatively small size and symmetrical shape. The plasma proteins that move most slowly in the electric field are the γ-globulins, which have nearly neutral isoelectric points and therefore low net charges at neutral and slightly alkaline reactions. Fibrinogen, although of far larger size and less symmetrical than the γ-globulins, has an acid isoelectric point and moves more rapidly under the standard conditions adopted. The a- and β-globulins of various size, shape, and isoelectric points move at decreasing rates of speed, in the order a_1-, a_2-, β_1-, β_2-, intermediate between those of albumin and fibrinogen. The a- and β-globulins in the various fractions generally represent quite different protein components. Albumins crystallized from human plasma have revealed components with different mobilities both in acid solution (80) and, following prolonged electrophoresis, in alkaline solution.[3] The electrophoretic heterogeneity of γ-globulins has also been recognized (82). The association of these variations in mobility with chemical and with immunological differences may be anticipated as our knowledge of these substances in normal and pathological conditions increases.

ULTRACENTRIFUGAL SEDIMENTATION

The speed of motion in the quasi-gravitational field of the ultracentrifuge, for the development of which we are indebted to Svedberg (83), depends on the size of the molecules, the difference between their densities and that of the solution, and their shape. The characterization of the molecule afforded by the ultracentrifuge is in terms of a sedimentation constant. Earlier workers (53, 84) found that plasma normally contained proteins sedimenting with four different velocities and that their relative quantities depended on dilution. The approximate quantities sedimenting with these velocities in almost undiluted and in highly diluted normal human plasma are recorded in the accompanying table. The constant for X-protein is listed as X,

[3] By Armstrong in this laboratory (79) and by Krejci and Sweeny (81).

since the density of this material is so near unity that its sedimentation rate varies greatly with the salt concentration.[4]

Component	Sedimentation Constant in Water at 20° C. $s_{20,w}$	Per Cent in Plasma	
		Undiluted	Diluted
Albumin (A)	4.6	65	80
Globulin (G)	7.1	10	15
X-protein	X	20	0
20-component	20.	5	5

The distribution of protein of these sedimentation constants in the plasma fractions is graphically represented in Figure 50. Fractionation has revealed material with sedimentation constants between 8 and 10 and between 11 and 18, somewhat arbitrarily divided into two groups with S-8 to 11 and 12 to 18, in Figure 50. The classification of plasma proteins by sedimentation does not correspond to that by electrophoresis, in that a fraction that appears homogeneous by one method may be separated into two or more fractions by the other. The number of components must therefore be greater than is indicated by either method alone.

Ultracentrifugal analysis was used at first as a criterion of purity of the γ-globulins (21), but was soon discontinued for that purpose because it appeared to agree less well than electrophoretic analysis with the important physicochemical and physiological properties of these materials. Ultracentrifuge studies have been most useful in following fractionation, however, and particularly so in detecting denaturation, since the formation of polymerized or degraded molecules is usually detected by this method.

OSMOTIC PRESSURE

The osmotic pressures of albumin solutions were studied thoroughly because of the importance of osmotic activity for their therapeutic uses (24, 74). The osmotic pressures of other components and fractions were investigated in the course of their physicochemical characterization. Since the ratio of osmotic pressure to concentration, after extrapolation to zero concentration, is directly proportional to the number of molecules in unit weight, osmotic pressure offers a direct measure of the molecular weight of a homogeneous protein. With a mixture, it determines the number average molecular weight, which is the total weight divided by the total number of moles. Since it gives no indication of homogeneity, it is not used to determine the extent of separation or purification.

The osmotic efficiency of a colloid may be defined as the volume of solu-

[4] The complications of sedimentation analysis are discussed in more detail in the thesis of Pedersen (85), which appeared after most of our work was completed, and in a paper from our laboratory (66).

tion held by 1 gm. of colloid at a given osmotic pressure. The osmotic pressure of normal human plasma is about 26 mm. of mercury at 37° C. At this pressure and temperature and at the pH and ionic strength of blood the osmotic efficiency of human serum albumin was found to be 18 cc. per gram. For plasma, the efficiency was found to be about 0.8 that of albumin, or 15 cc. per gram at this pressure. On the basis of these results, 100 cc. of 25 per cent albumin was recommended as osmotically equivalent to 500 cc. of 6 per cent plasma (24).

The contribution of the various fractions to the osmotic activity of plasma is shown in Figure 50. At very low pressures, the sum of the volumes of the fractions was equal to that of the plasma, suggesting that the total number of molecules was not changed by fractionation. At the pressure of plasma, the sum of the volumes of the fractions was somewhat less than that of the plasma, which may reflect experimental error or interactions between the proteins.

VISCOSITY

The viscosity of a solution determines the ease with which it will pass through a hypodermic needle or a filter pad. The concentrations in which it was convenient to distribute the various fractions were often limited by the viscosities of the solutions. Like osmotic pressure, viscosity measures an average property of all the molecules without distinguishing between the components of a mixture, so that it is not very useful in following fractionation. Independent of the size of the molecules, and varying only with their shape and degree of hydration, viscosity does give a valuable additional method of characterizing the separated components of plasma.

The contribution of the fractions of plasma to the viscosity is also represented graphically in Figure 50. The sum for the fractions is slightly greater than that for plasma, but the difference may be no greater than the experimental error. Figure 50 reveals that the contribution of Fraction V to the osmotic activity is greater than its fraction by weight, and that its contribution to the viscosity is much less. At the other extreme is Fraction I, whose contribution to the osmotic pressure is less than its weight fraction, while its contribution to the viscosity is very much greater.

LIGHT SCATTERING

In dilute solutions, the scattered light, or the turbidity, is proportional to the concentration of scattering particles, but it depends greatly on differences between their indices of refraction and that of the solution and on their size. The light scattered per unit mass is greatest for particles whose diameter is equal to the wave length of light, about 5000 Å, and is very small

for particles less than one-tenth or more than ten times this wave length. Therefore, the measurement of light scattering has not been important in following fractionation, but it has been very useful in determining denaturation, for which it was first suggested by Mr. Peter Morrison. An instrument was devised especially for this purpose by Dr. Mueller.[5] Measurement of light scattering has proved invaluable for laboratory studies and has been adopted for measurements of the stability of albumin solutions (23). Light scattering even in the range below visibility is readily revealed by the nephelometer, and is the basis on which albumin has been accepted for the Navy.

PHYSICAL PROPERTIES OF COMPONENTS

Certain plasma proteins have been sufficiently purified by fractionation and subfractionation so that the physical properties of the homogeneous components can be estimated. These properties are recorded in Table IV. The components whose molecular weight estimates are enclosed in parentheses have been studied only as minor components of fractions, whereas the others have been isolated in moderately pure states. The properties listed are extrapolated values estimated for the pure substances. The third column gives the approximate amount of the component estimated to be in normal pooled human plasma. About 80 per cent of the protein is accounted for by these components. The remaining 20 per cent represents in part other components, and in part these components distributed in small amounts in other fractions. The fourth column gives the sedimentation constant, $s_{20, w}$ and the fifth one hundred times the intrinsic viscosity, H_0.[6] The sixth column gives the molecular weight, and the seventh and eighth the length and diameter calculated on the assumption that the molecules are ellipsoids of revolution. These quantities are estimated from the sedimentation, viscosity, osmotic pressure, and diffusion constants and from double refraction of flow, and depend somewhat on the assumptions as to the amount of hydration. The β_1-lipoprotein appears to be spherical, and most other components asymmetric but with the same diameter (35–50 Å) regardless of their length (Fig. 51). This dimension is presumably critical for the retention of plasma proteins in the blood stream. It may be compared with the much smaller diameters of sodium (1.9 Å) and chloride (3.6 Å) ions, of water (3.9 Å) and glucose (*ca.* 7 Å) molecules, and of gelatin (18 Å). It may also be compared with the dimension of a red blood cell, which may be taken as a disk with a mean diameter of 76,000 to 86,000 Å (7.6 to 8.6 μ) and a thickness of 20,000 to 26,000 Å and has been estimated to contain 300,000,000 hemoglobin molecules.

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[5] See page 379.

[6] H_0 is the extrapolated value at zero concentration of $(\ln \eta/\eta o)/c$, in which η is the viscosity of the solution and ηo is that of the solvent.

TABLE IV

Protein Components of Normal Human Plasma Characterized by Physical Chemical Methods *

Electrophoretic Component	Fraction	Approximate Amount in Plasma (gm./l.)	Sedimentation Constant $s_{20,w}$	Intrinsic Viscosity $H_0 \times 10^2$	Molecular Weight M	Approximate Dimensions in Å†	
						Length	Diameter
Serum albumin	V	32	4.6	4.2	69,000	150	38
α₁-globulin ‡	IV-1	2	5.	6.6	200,000	300	50
α₂-globulin	IV-6	1	9.	9.2	(300,000)	—	—
β₁-globulin	IV-7	2	5.5	5.5	90,000	190	37
β₁-globulin	III-0, III-2	2	7.	—	(150,000)	—	—
β₁-globulin	III-0	1	20.	—	(500,000)	—	—
					1,000,000		
β₁-globulin ‡	III-0	2	2.9	4.1	1,300,000	185	185
β₂-globulin	III-1	2	7.	—	(150,000)	—	—
γ-globulin	II	5	7.2	6.	156,000	235	44
γ-globulin	II	1	10.	—	(300,000)	—	—
Fibrinogen	I-2	2	9.	25.	400,000	700	38

* Taken from Oncley, J. L., Scatchard, G., and Brown, A., *J. Phys. & Coll. Chem.*, **51**, 184, 1947.

† These estimates of approximate dimensions assumed an elongated ellipsoidal shape, and 0.2 gm. of water of solvation per gram of protein for all components except the β₁-lipoprotein, for which spherical shape and a solvation of 0.6 gm. of water per gram of protein were assumed.

‡ These two globulins are lipoproteins containing 35 per cent lipid for the α₁-globulin and 75 per cent lipid for the β₁-globulin. Osmotic pressure studies of these components have yielded apparent molecular weights lower than the values recorded here, and considerable leakage through the membranes used, which are tight to most plasma components. This result may be explained by assuming an equilibrium between some of the lipid and the lipoprotein.
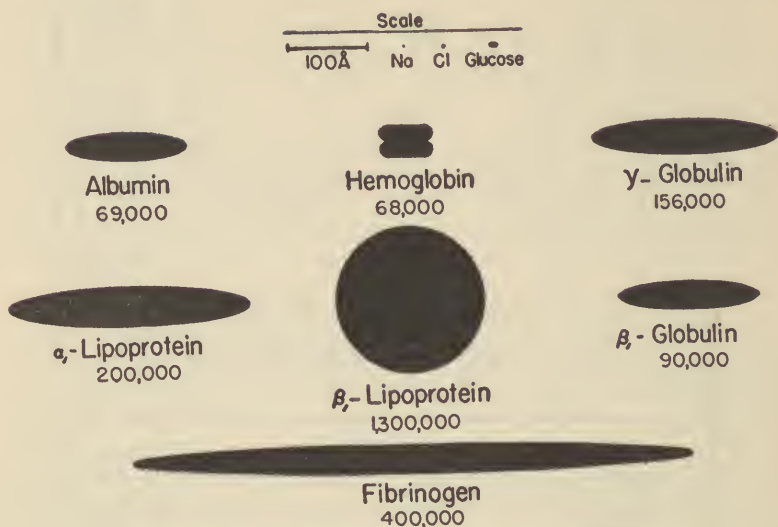
FIGURE 51. *Relative dimensions of various proteins.*
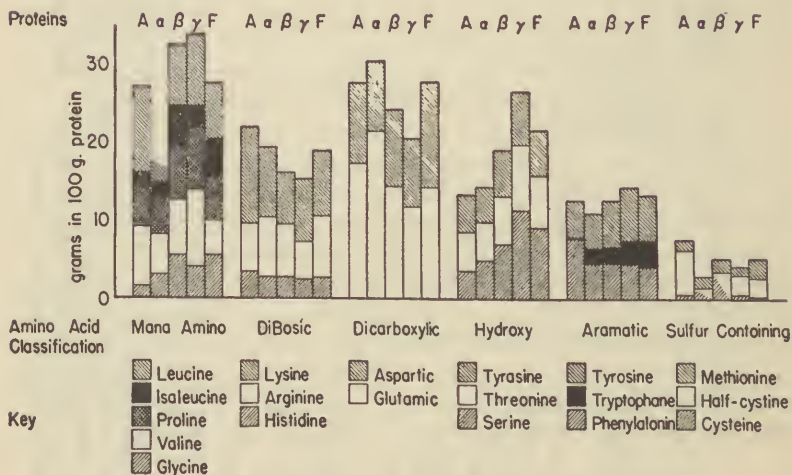
FIGURE 52. *Amino acid composition of various proteins from human plasma.*

Amino Acid Composition

The physicochemical properties of a protein depend on its amino acid composition. The analytical studies, carried out by Dr. Erwin Brand and his associates (22, 86) on the plasma protein fractions separated, revealed striking differences, which are graphically represented in Figure 52. Thus, the high content of hydroxyamino acids — tyrosine, serine, and threonine — in γ-globulin and in fibrinogen, F, contrasts strikingly with the relatively low content of these amino acid residues in albumin, A. Albumin also has a low glycine content and a still lower content of isoleucine and tryptophane. Characteristic differences have also been observed in the various α- and β-globulins separated in the subfractions of Fractions III and IV.

These analytic results on amino acids, as well as those on the carbohydrates and lipids associated with various plasma proteins, have aided in designing and controlling fractionation procedures. In connection with nutritional studies, thus far completed only in animals (40, 41), they have suggested that albumin is deficient in isoleucine and tryptophane.

Amino acid analyses have revealed differences not only in the composition of the various proteins separated from human plasma but also in the comparable protein, indistinguishable electrophoretically and in the ultracentrifuge, separated from human and animal plasma. Thus, the tryptophane content of the albumin crystallized from bovine plasma is roughly three times and that crystallized from horse plasma twice that of crystallized human serum albumin (22, 86). Such differences in the fine structure of proteins of the same size, shape, and net charge are presumably the basis of immunological specificity and of the solubility differences on which the fractionation process depends.

V. NORMAL HUMAN SERUM ALBUMIN

Shock

The first clinical studies with normal human serum albumin were aimed at defining its physiological action and value in the treatment of shock. Since it was prepared in concentrated solution for convenience in shipping and storage under military conditions, it was first necessary to prove that its injection in large amount was followed by no untoward reactions and resulted in a shift of fluid across the capillary membrane, just as occurred across the artificial membrane in an osmometer. This was demonstrated by Stead and Ebert (15) (Fig. 53). Experiments were next carried out to determine the quantitative response to a given dose of albumin. After removing a measured amount of blood from human subjects, the increase in plasma volume that followed the injection of a known amount of concentrated

serum albumin (either human or bovine) was determined. In eleven experiments with volunteers, most of whom were Harvard Medical School students, the average increase one hour after injection was found to be 17.4 cc. per gram (16, 87), as compared with the 18 cc. per gram (24) predicted
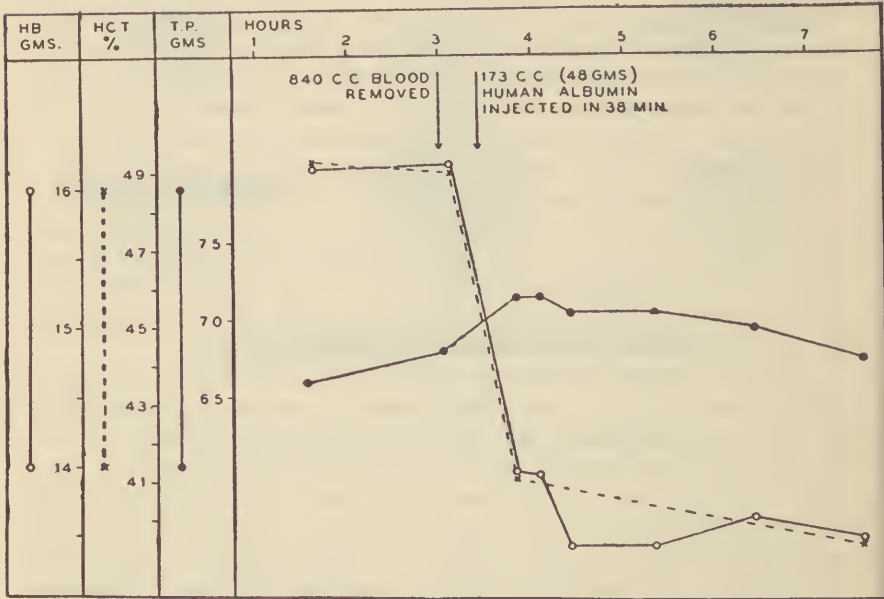
FIGURE 53. *Changes in the values for the hematocrit, hemoglobin and plasma protein (falling drop method) in a normal human subject, following a large venesection and the subsequent administration of a concentrated human albumin solution.*

from laboratory measurements. With such good agreement between results obtained in vitro and in vivo, clinical trial of this new product was placed on a firm quantitative basis.

The stability of albumin permitted its being dispensed in solution. Its low viscosity permitted a concentration of 25 gm. in 100 cc., or five times that in plasma. This volume was selected as the unit on the basis of reports of the large amounts of plasma being used by the British in the field in the treatment of shock. It was osmotically equivalent to approximately 500 cc. of citrated plasma, or to two of the dry plasma units being prepared at that time. The large dry plasma unit introduced thereafter, however, was osmotically equivalent to the albumin unit, and therefore they could be used interchangeably. An extremely convenient package was developed by Com-

mander, later Captain, Newhouser of the Navy and Major, later Colonel, Kendrick of the Army (88, 89), which weighed roughly one-sixth as much as the osmotically equivalent package of dried plasma and occupied one-sixth as much space. This completed preparations for the military use of albumin.

Clinical experience in a number of centers (see footnote 17, page 374) demonstrated that albumin could be safely and effectively used in cases of hemorrhage, trauma, and burns (Fig. 54) to produce an increase in plasma volume and to bring about clinical improvement. The initial evaluation of
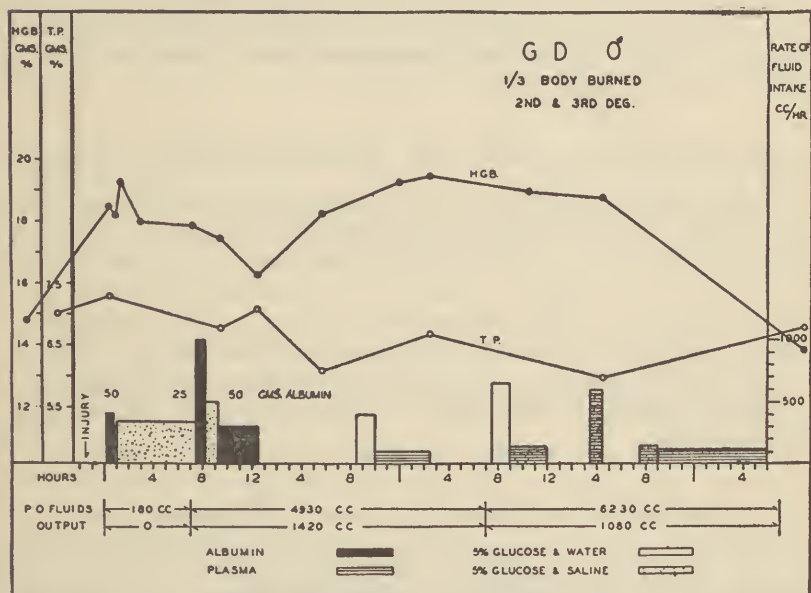
FIGURE 54. *Use of concentrated albumin (total of 125 grams) in conjunction with 5 per cent glucose and physiological saline solution to control hemoconcentration (shown by HGB) and maintain plasma protein concentration (shown by T.P.) during the first 14 hours after a severe 30 per cent burn.*

albumin in the treatment of shock, which led to the recommendation of this blood derivative to the armed forces in January 1942, culminated in a report with instructions for its use in May 1942 (90, 91).

Because of the urgent need of the armed forces for as much albumin as possible, clinical investigation was thereafter restricted to studies that could be made in the course of testing the safety of the large number of lots

of material being produced under Navy contract, each of which was administered to several patients before release. By the selection of suitable patients with diseases such as cirrhosis of the liver, the effect of human albumin on hypoproteinemia was explored and its safety when administered repeatedly over long periods established (25).

Clinical shock has since been critically studied (2, 26) by two groups, in New York under Drs. Richards and Cournand, and in Atlanta under Drs. Stead and Warren. They confirmed the effectiveness of albumin in increasing plasma volume in shock and added important information concerning its hemodynamic effects, particularly the increase in cardiac output that followed its use. Their continued collaboration made it possible to authorize changes in diluent and method of preparation, after suitable field trials, as technical improvements were introduced.

Problems Encountered in the Adoption of Serum Albumin

Certain possible dangers in the use of concentrated serum albumin in the treatment of shock were always recognized. The first was the danger, or ineffectiveness, of using a concentrated solution to treat a dehydrated patient. The second was the danger of producing a deficiency of the various functionally important globulins by replacing lost blood or plasma with serum albumin. The third was the danger of producing serious damage in kidneys, already suffering from the reduced blood flow of shock, with the mercurial preservative specified in the minimum requirements of the National Institute of Health.

Concentrated Serum Albumin in Dehydration

In the experiments on volunteers (16), an attempt was made to determine whether the increase in plasma volume after injection of concentrated albumin was less in subjects with mild dehydration than in subjects who had been given large quantities of salt and water by mouth. No appreciable difference was found. As an index of damage to cells from the injection of a hypertonic solution, serum potassium and potassium excretion in the urine of these subjects were studied by Dr. James L. Gamble (16). No significant increases in serum level or urinary output of potassium occurred.

Bovine serum albumin was used in a number of physiological studies on experimental shock aimed at a solution of this problem (92, 93, 94). These may be summarized as indicating that concentrated serum albumin was not harmful *per se* in forms of shock associated with dehydration, but was less effective until supplemented with additional saline. This was particularly demonstrated in animals with tourniquet shock by Fine and his associates.

Because conclusive clinical evidence on the possible harmful effects of

albumin could not be obtained in the original evaluation, a precaution urging the use of additional fluids in all cases with dehydration was etched on every bottle. Nonetheless, a report from the Mediterranean Theater indicated that 100 cc. of concentrated 25 per cent albumin was only half as effective as 500 cc. of unconcentrated plasma (95). Additional fluids had not been used and the comparison of equal volumes of 5 per cent (isotonic) albumin and plasma had not been made. Further trials of albumin in shock were therefore undertaken at this time at the request of the Navy, and these were conducted under the auspices of the Committee on Medical Research by four teams working on shock and directed by Dr. D. W. Richards, Jr.[1] Study of their reports indicates that concentrated serum albumin produced in most shock cases an immediate increase in plasma volume averaging 10 to 12 cc. per gram of albumin injected, with considerable variation from case to case, and that the full increase of approximately 18 cc. per gram was obtained when additional fluids were administered, as suggested in the original directive (see Table V and 96, 97). The fact that 25 per cent albumin, unlike concentrated plasma, is no more viscous than whole blood probably explains the absence of harmful effects from its use even in dehydrated patients.

INFLUENCE OF INJECTION OF SERUM ALBUMIN ON GLOBULINS

It was feared that administration of albumin might lead to globulin deficiency, particularly in burns, where plasma loss is rapid and replacement dosage large. Important serum globulins, prothrombin and fibrinogen, are essential for blood coagulation, while antibodies and complement are needed for an intact immune mechanism. Studies were made on the latter in dogs given bovine albumin (68, pages 197–198) and on prothrombin and total serum globulin in certain patients. Under ordinary circumstances, no evidence of globulin deficiency could be found. In one or two cases, when albumin was used continuously in massive doses globulin deficiency was manifested by low serum globulin, and a prolonged prothrombin time with an increased tendency to oozing, which was readily corrected with plasma (25, 90, 91).

DANGER OF MERCURIAL PRESERVATIVES

Apprehension concerning the effect of mercurial preservatives in plasma or albumin on kidneys already damaged by the ischemia of shock was voiced by Dr. Weiss and others early in the deliberations of National Re-

[1] The groups co-operating under Dr. Richards were as follows: New York City, Drs. A. Cournand and A. Lowell; Atlanta, Drs. E. A. Stead, Jr., and J. V. Warren; Philadelphia, Dr. J. Rhoads; Richmond, Dr. E. I. Evans.

search Council committees. The extent to which such fear was justified has never been adequately determined. Together with the fear of red blood cell and globulin deficiency, which might result from overadministration of plasma or albumin to acute shock cases, it led to limitation of the dose to 10 units or 250 gm. of albumin in forty-eight hours. The practice of using a preservative was discontinued with the introduction of salt-poor albumin (see page 381).

TABLE V

Effect of Concentrated Albumin in Shock *

Plasma Volume Increase Observed in Patients With Measurements Made Within 1½ Hours of Injection of Albumin

Type of Case	No. of Cases	Plasma Volume Increase cc./gm. of injected albumin	
		Spread	Average
Predicted from osmotic pressure measurements (24)			18.0
Experimental hemorrhage in man (16)	11	13.2–24.1	17.4
Clinical shock (2) †			
(No additional saline or small amount)	63	0–31.7	11.7
Clinical shock (2) †			
(Additional saline)	20	7–29	17.9

* Published also in Janeway, C. A., *Ann. Int. Med.,* 26, 368, 1947.
† Figures derived from published tables (2), and from report of study conducted by Dr. D. W. Richards, Jr. (See footnote 1, above.)

SALT-POOR ALBUMIN

Interest in the use of albumin in the treatment of hypoproteinemic edema, particularly in renal disease, stimulated the development of salt-poor albumin (18), which was made possible by the studies of the stabilization of albumin solutions. Albumin is so stable that it alters only slowly even when subjected to high temperatures. It then becomes more viscous, at first slowly but then much more rapidly. At about the time that the viscosity begins to increase rapidly, a haze develops (see page 399). A crystalline albumin in 0.15 M sodium chloride at pH 6.8 developed a visible haze in about six months at 50° C. (112° F.). Gel formation occurred only after an interval roughly three times that for the formation of the first visible haze. As the temperature increased the speed of each of these reactions also increased, and was approximately doubled for each degree centigrade increase in temperature.

At the time of the first large-scale production of albumin it was known that the stability, maximum at pH 6.8, was nearly proportional to the salt

concentration. The original specifications for a 25 per cent solution of albumin were altered in March 1943 from 0.15 M (isotonic) to 0.3 M sodium chloride in order to increase stability.

It has been shown by Dr. Luck and his co-workers at Leland Stanford University that many organic anions are much more effective stabilizing agents for albumin than the chloride ion (17, 72), and their efficiency has been correlated, with a few exceptions, with the extent of complex formation between the albumin and anion (98).[2]

These discoveries became especially important when albumin containing no mercury and low in sodium was desired as a diuretic agent. The 1945 Navy specifications for albumin to be used either as a diuretic agent or for the treatment of shock call for a 25 per cent solution of albumin, at pH 6.8 ± 0.2, stabilized with 0.04 M per liter of sodium acetyltryptophanate,[3] without mercurial but heated for ten hours at 60° C. in the final container. Even after this heat treatment, the albumin has a higher stability than unheated albumin stabilized with 0.3 M sodium chloride (18). The sodium content is less than a fifth of that of the osmotically equivalent quantity of normal plasma. There is about 0.1 per cent of sodium ion to neutralize the albumin, 0.0 to 0.1 per cent to neutralize absorbed acids, probably mostly acetic acid, and 0.1 per cent in the acetyltryptophanate.

Heating of albumin in the final container for this period of time has been demonstrated by Drs. Gellis and Stokes to bring about inactivation of the virus of homologous serum hepatitis (19). If a higher temperature were found desirable to destroy some other virus or bacteria, a mixture of 0.02 M

[2] This stabilizing action is very specific. These sodium salts do not affect the stability of γ-globulin, which is, however, markedly stabilized by many sugars and by glycine. Hence, when it is used for intramuscular injection, 0.3 M glycine, which is approximately isotonic, is the recommended stabilizing agent for γ-globulin.

[3] The various steps that led to the adoption of this stabilizing agent illustrate beautifully the interplay of ideas in this research. The action of lactate and succinate was being considered by groups studying shock on the basis of animal experiments; by Dr. Winternitz (Yale University) and Dr. Shorr (New York Hospital). If metabolized anions were valuable it seemed desirable to omit the reprecipitation of albumin to free it of acetate, and the Harvard Pilot Plant prepared albumin that had not been reworked in order to remove acetate. Dr. Luck (Leland Stanford University) found that acetate ion gave a greater stability than the same amount of chloride ion; Dr. Edsall (Harvard University) suggested trying propionic acid; Dr. Luck found that this was more efficient, and that longer-chain aliphatic acids, from butyrate to caprylate, and some aromatic acids were very much more efficient, and recommended phenylacetate or mandelate; Dr. Clarke (Columbia University) suggested the sodium salt of acetyl phenylalanine as more closely related to the natural constituents of the plasma; Dr. Strong (Harvard University) suggested the salts of the acetyl derivatives of the amino acids in which albumin is deficient for nutrition (tryptophane and isoleucine). The salt of acetyl-isoleucine is not very effective, but sodium acetyltryptophanate is effective. The combination of stabilizing efficiency, safety, and possible value in nutrition led to its being the first of the stabilizing agents to be accepted. Dr. Scatchard (Massachusetts Institute of Technology) suggested that the greater stability would permit a heat treatment in the final container, which might make unnecessary the addition of a bacteriostatic agent, and might destroy virus.

sodium acetyltryptophanate and 0.02 M sodium caprylate could be heated for ten hours at 63° C. without undue loss of stability. Proof that salt-poor albumin was as satisfactory for the treatment of shock as the albumin solutions of higher salt content was rapidly obtained with the collaboration of our associates (99, 100). This very satisfactory product, rich in protein and poor in electrolyte, therefore replaced the earlier standard albumin solution in the Navy contracts of 1945.

Albumin in Hypoproteinemia

The original exploration of the field of chronic hypoproteinemia (25) demonstrated that albumin was not excreted in the urine by patients with normal kidneys, that nitrogen was retained in cases of hypoproteinemia, but that large repeated doses were needed to restore a diminished level of serum albumin to normal.

In the *nephrotic syndrome,* albumin has had a limited trial.[4] Its administration is followed by transient increases in the serum albumin fraction (102), markedly increased excretion of albumin in the urine, and, in many, but not all cases, a diuretic response (46, 101). It is difficult to produce a sustained increase in the serum albumin level in adults with doses of less than 50 gm. daily for several weeks (103). Diuresis with albumin differs from the spontaneous type of diuresis in its direct correlation with albumin administration and its more moderate extent. The safety of albumin and its low salt content warrant further clinical studies. Its use has helped to delineate the problem of nephrosis. The fact that a marked increase in albumin excretion follows the administration of normal human serum albumin suggests that the nephrotic kidney cannot retain normal albumin molecules, while the failure of temporary increases in colloid osmotic pressure to effect a diuresis in all cases is further evidence of the disturbed relationships involved in the control of water balance in these patients. Such findings serve to emphasize the value of plasma fractions as tools for the study of disease processes.

In hepatic disease albumin has a very specific role, since hypoalbuminemia occurs with diminished liver function and, in the absence of proteinuria, elevation of the serum albumin level can be accomplished much more quickly than is possible in the nephrotic syndrome. The use of albumin in cirrhosis has been investigated in a few cases (25, 47), and its effectiveness in the treatment of hepatitis is under study (104, 105). In hepatic disease the role of reduced colloid osmotic pressure in the production of edema and ascites has been further defined by noting the effect of restoring the serum albumin level to normal by intensive replacement therapy.

[4] The superiority of salt-poor albumin over the earlier salt-containing albumin in this condition has not been conclusively proved, though the existing evidence is highly suggestive (Fig. 55) (101).

In surgical patients with hypoproteinemia, albumin also appears to be a specific agent to restore osmotic equilibrium to normal, and this problem is under study (106). Results in certain surgical patients with low serum proteins, a poor state of the peripheral circulation, edema, and oliguria have

FIGURE 55. *Changes in body weight of a three-year-old child with nephrosis. Compare response to daily injections of salt-poor albumin (0.3 gm. of Na^+ per 100 cc.) (barred lines) with that to standard albumin (1.0 gm. of Na^+ per 100 cc.) (solid lines).*

been dramatic. The compactness and ease of administration of albumin make it particularly suited to the treatment of hypoproteinemia in infants and children (Fig. 56) (101, 107).

A number of possible uses for concentrated human serum albumin in the treatment of generalized as well as local edema remain to be studied. The availability of a concentrated solution, low in sodium, of the plasma component most active in maintaining normal osmotic relationships and the one most frequently diminished in protein deficiency with edema provides a solution for parenteral use, which, with solutions of glucose, electrolytes, and amino acids, should make it possible to rectify most disturbances of fluid balance. Its usefulness in treatment and in the study of physiological disturbances in diseases such as nephrosis, cirrhosis of the liver, and osteoporosis (108) is being demonstrated in studies now in progress.
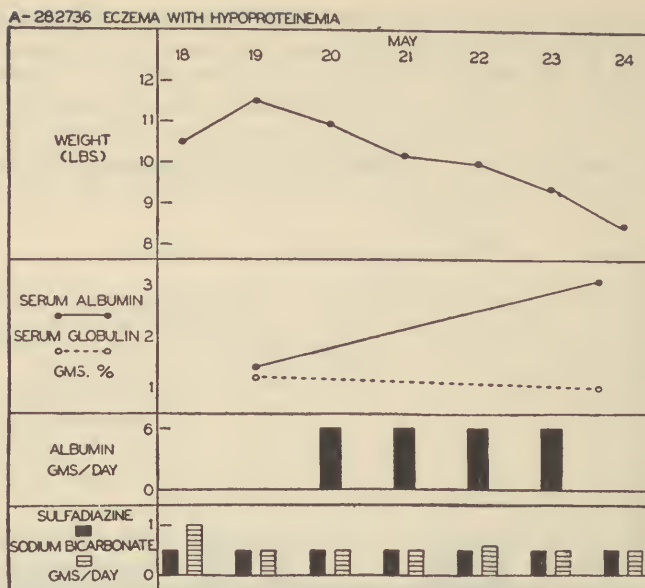
FIGURE 56. *Clinical course of a baby with severe hypoproteinemia, due to weeping infected eczema, who was given concentrated human serum albumin 6.25 gm. (25 cc.) per day for four days. On May 19 the baby had severe anasarca and ascites, while on May 24 only slight edema remained about the area of infection on the forehead and scalp.*

VI. SERUM γ-GLOBULIN

MEASLES ANTIBODIES IN FRACTION II + III

Very early in the program for fractionating plasma, it was suggested by Dr. Elliott Robinson (13, page 3398, footnote) and by Dr. Joseph Stokes, Jr. (see page 370) that the globulin fraction might be used for the control of measles and other infectious diseases. Laboratory studies soon demonstrated that the measurable antibodies in pooled plasma could be quantitatively recovered in Fraction II + III. During late 1941 and early 1942 Mr. Marshall Melin developed a dialysis procedure (Globulin Method 1), carried out just above the freezing point of the solvent, which yielded solutions that could be clarified and sterilized by filtration through Seitz-type filter pads, were almost uniformly free from substances causing pyrogenic reactions in rabbits, and contained but little thrombin and prothrombin. The antibodies

in these preparations were concentrated eight- to ten-fold over plasma, as was demonstrated by the antibody survey conducted by Drs. Dochez, Enders, and Boyd (Table II).

In the winter of 1942–1943 Dr. Stokes and his associates of the Commission on Measles and Mumps of the Army Epidemiological Board, with the aid of a number of physicians in the Philadelphia area (28), demonstrated that this material was very satisfactory in relatively small doses for passive immunization against measles.

Measles Antibodies in Fraction II

Procedures for the subfractionation of Fraction II + III were meanwhile being developed under the direction of Dr. J. L. Oncley. This was desirable because the dialysis procedure was complicated and not easily adapted to large-scale production, the thrombin and prothrombin from this fraction were needed for blood clotting, and the isoagglutinins were needed for blood grouping. In addition, other materials of potential usefulness, present in this fraction, included plasminogen, a number of enzymes, and large amounts of β-lipoprotein that could not be dried from the frozen state. Lipoproteins and enzymes lead to instability in antibody preparations.

The first of a series of subfractionations that yielded a highly purified, stable, more concentrated preparation of the antibodies (Method 2) was completed on June 15, 1942. At this time more systematic antibody tests were initiated by Dr. Enders, in order to control as precisely as possible the antibody titer of the preparations made in commercial laboratories. These tests are being continued. The use of a reference standard to increase the reproducibility of these tests was introduced during the fall of that year. Besides electrophoretic analysis for γ-globulin, the following antibodies have been assayed:

On All Preparations

Agglutinins against typhoid O antigen
Agglutinins against typhoid H antigen
Antihemoagglutinin against influenza A virus, strain PR 8
Neutralizing antibody against influenza A virus, strain PR 8 (mouse protection test)
Diphtheria antitoxin (interdermal neutralization test on rabbits)

On Most Preparations

Complement-fixing antibody against the virus of mumps
Complement-fixing antibody against influenza A virus, strain PR 8

On Selected Preparations

Agglutinins against H pertussis, phase 1, strain 484, Phila.
Streptococcal antitoxin (interdermal neutralization test on rabbits)
Antistreptolysin O

Of these antibodies, all but the agglutinins against typhoid O antigen and the isoagglutinins, both present in Fraction III–1, were found concentrated

in Fraction II. By making a solution of Fraction II containing 16.5 gm. of
γ-globulin per 100 cc., a preparation having twenty to twenty-five times the
antibody titer of the original plasma has been routinely achieved.[1] The titer
of various preparations, even when obtained from large pools of normal
human plasma, was found to vary over a considerable range. This variation
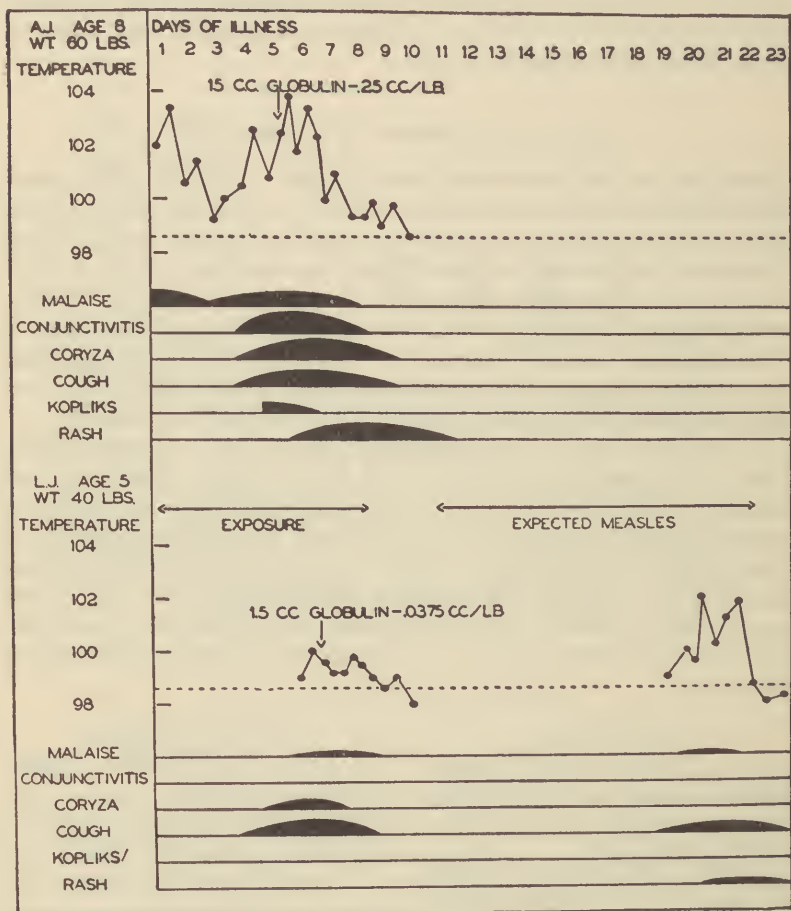
FIGURE 57. *Satisfactory modification of measles in a child (lower
graph) intimately exposed to an older sister. Note lack of re-
sponse to a much larger dose administered to the older child
(upper graph) just before appearance of rash.*

[1] A 25-fold concentration would be expected on the basis of γ-globulin concentration,
since normal human plasma contains 6.6–7.2 gm. of γ-globulin per liter.

is almost certainly due to the variation of antibody concentration in the plasma pools, due in part to the geographical location of the area from which the pool was obtained and in part to seasonal variations in antibody levels (27).

The purity of the γ-globulin and the amount being prepared in the Harvard Pilot Plant increased greatly during 1942. Method 3 was introduced in October 1942 and on March 22, 1943, the γ-globulin concentrated in Fraction II was recommended to the armed forces by the National Research Council for measles prophylaxis.

The spring of 1943 was marked by severe measles epidemics in Philadelphia, Baltimore, and Boston, and as Fraction II, with its greater concentration of γ-globulin, was developed, there was ample opportunity to prove its value. The Philadelphia and Baltimore groups (28) established human serum γ-globulin as an effective agent for the prevention or modification of measles when administered in the first half of the incubation period. They also felt that when it was injected in much larger doses, even after the development of early symptoms, it diminished the severity of the disease. In general, however, results in the treatment of established measles have not been striking. In Boston the value of γ-globulin in the prevention and modification of measles was confirmed in a small, carefully controlled study and in a group of cases treated by practicing physicians (29). On the basis of this evidence and the record of approximately 150,000 cases of measles among troops in Army camps in the United States in 1917–1918, contracts were made for commercial production of γ-globulin.

To assure satisfactory material under these contracts, specifications for antibody content were established on the basis of Dr. Enders' assays, and, since no specific laboratory tests for measles were available, an attempt was made to test the potency of each preparation by field trials. These were carried out with the co-operation of many physicians in the United States, Great Britain, and Australia.[2] To date, forty-seven preparations of γ-globulin have been tested and reports of over three thousand injections analyzed. The results have been quite satisfactory. Reactions have been very few, homol-

ogous serum jaundice has not occurred, and complications in modified cases of measles have been almost nonexistent (107). These tests have proved that, provided γ-globulin is administered within eight days of exposure, the results will depend on the dose. Thus, an intramuscular injection of 0.1 cc./lb. has been established as the protective dose and 0.025 cc./lb. as the modifying dose. The results in children exposed at home, with an expected attack rate of 75–85 per cent, are cited in Table VI.

TABLE VI

Relation of Dose of γ-Globulin to Results *

(1024 cases given globulin within 8 days of exposure)

	No Measles	Mild Measles	Average Measles	Attack Rate
	%	%	%	%
		Untreated Measles		
Boston 1943 (29)	17	4	79	83
New York 1944 (110)	20	33	47	80
Dose (cc./lb.)		Cases Given γ-Globulin		
0.01–0.0375	33	63	4	67
0.0375–0.075	55	41	4	45
0.075–0.2	84	15	1	16

* Taken from Janeway, C. A., *Bull. New York Acad. Med.*, 21, 202, 1945.

The importance of a large-scale public health experiment with γ-globulin was first stressed by Dr. William Thalhimer, then Director of the Manhattan Convalescent Serum Laboratory. His previous studies on measles and its control in New York City provided a baseline for our evaluations. A study such as he urged was carried out by the Department of Health of the City of New York in co-operation with the American Red Cross in 1944, together with a comparison of γ-globulin and globulin derived from human placentas (110). Not only was the particular placental preparation they used less effective, but it produced nearly fifty times as many reactions. With the value of γ-globulin proved from the public-health standpoint, a very important step was taken in 1945, when the American Red Cross undertook to return to the people such amounts of γ-globulin as were in excess of the needs of the armed forces (111).[3]

[3] This policy was suggested in a letter to Dr. Lewis Weed as newly appointed Chairman of the Medical Advisory Council of the American Red Cross, on April 6, 1943. Soon thereafter a memorandum on "The Control and Production of By-Products from the Blood Collected for the Armed Forces by the American Red Cross" prepared at the request of and following a meeting of this council was submitted to them with the endorsement of the Subcommittee on Blood Substitutes of the National Research Council. The wise policy inaugurated with γ-globulin is now being followed with other blood derivatives, which are being decreed surplus at the end of the war and made available to the public by the American Red Cross through public-health agencies,

INFECTIOUS HEPATITIS

Infectious hepatitis proved to be an extremely serious problem among troops in World War II. No method of controlling this disease was available; hence, the importance of the finding by Stokes and Neefe (112) that it could either be prevented or modified in exposed persons by the injection of γ-globulin. Stokes and Gellis were requested to see whether these results could be duplicated in the Mediterranean Theater, where in 1944 and 1945 infectious hepatitis was a serious problem. The protective value of γ-globulin in this disease was reported from studies in Italy (113), but its value in the treatment of hepatitis (114) could not be established. Subsequently Havens and Paul (115) demonstrated the usefulness of γ-globulin as a means of passive protection during an epidemic in an institution for children (Table VII).

HOMOLOGOUS SERUM JAUNDICE

Although the evidence appears to be satisfactory that γ-globulin can be used to control epidemic infectious hepatitis, a more difficult problem remains in homologous serum jaundice, characterized by its occurrence several months after transfusion and by the fact that it can be transmitted only by parenteral inoculation and not by ingestion of infected serum. Stokes (116) has attempted to study this problem in battle casualties returning to Army general hospitals in this country. The results in one hospital were encouraging, but have not been consistent in others. Further studies are being carried out.

MUMPS

The antibodies against mumps in pooled normal human plasma are concentrated in Fraction II as judged by complement-fixation (27). Although concentrated twenty-fold with respect to the plasma, the use of this concentrate in the prophylaxis or treatment of mumps has thus far proved completely ineffective (117).

In mumps and scarlet fever, unlike measles and poliomyelitis, in which the antibodies are reported to be elevated in convalescence only a few fold over normal adult levels, they are elevated ten- to thirty-fold. The possibility of preparing far more concentrated antibody preparations against mumps and scarlet fever is therefore far greater if convalescent rather than pooled normal plasma is fractionated.

Fractionation of mumps convalescent plasma was carried out in the Harvard Pilot Plant with material collected by the Commission on Measles and Mumps during an outbreak in the Army. The globulin derived from this

TABLE VII[*]

Summary of Results with γ-Globulin in Prevention of Infectious Hepatitis

Source of Study	Given Globulin			Controls		
	Number of Cases	Probable Hepatitis	Jaundice	Number of Cases	Probable Hepatitis	Jaundice
		%	%		%	%
Stokes and Neefe, U.S.A. (112) (Pennsylvania)	53	21.0	6.0	278	67.0	45.0
Gellis, Stokes, Brother, Hall, Gilmore, Beyer, and Morrissey (113) (Mediterranean Theater of Operations)	1) 406	—	1.0	683	—	3.7
	2) 831	—	0.4	8270	—	3.4
	3) 495	—	0.4	1373	—	2.0
Havens and Paul (115) (Connecticut)	97	8.0	2.0	155	34.0	23.0

[*] Composite table from data much of which is also reviewed by Stokes, J., Jr., *Ann. Int. Med.*, 26, 353, 1947.

material proved to have ten to fifteen times more complement-fixing antibody than normal human serum γ-globulin. It was thus two hundred to three hundred times as concentrated with respect to this specific component as normal plasma. Although its prophylactic value has not been tested, a significant reduction in the incidence of orchitis when it was administered on the first day of mumps parotitis indicated that it contained sufficient antibody to affect the virus (118).

Scarlet Fever

Human convalescent serum has been recommended by certain physicians for the prevention and treatment of scarlet fever, a disease to which adults are generally immune and in which, as in mumps, the antibodies are elevated in convalescence. The importance of hemolytic streptococcal infections in Army camps and naval training stations in this country prompted Dr. A. R. Dochez to urge a study of the use of γ-globulin, since it contained a higher concentration of scarlatinal antitoxin than does convalescent serum. Studies were initiated in several clinics in 1944 and are still continuing with γ-globulin (Fraction II) supplied by the American Red Cross. Because of the complex and variable nature of this disease, no final conclusions on its value can yet be made.

Several pools of convalescent plasma have been fractionated.[4] γ-globulin with a far higher antitoxin content (140–280 units per cc.) than that in γ-globulin from normal plasma (30–50 units per cc.) was thus obtained. However, despite the pooling of bloods from one place, where over 90 per cent of cases were due to a sulfadiazine-resistant type 17, group A streptococcus, the concentration of type-specific antibacterial antibody was very disappointing. Dr. Homer Swift has suggested that the low titers obtained were probably attributable to the early stage of convalescence when the blood was collected. This material was turned over to the Navy for special studies.

Other Diseases

Trial of γ-globulin in other diseases to which adults are immune has revealed a number in which it appears to be ineffective (see Table VIII). These include prevention of chicken pox and infantile diarrhea (119). Routine administration of globulin to alternate premature babies in a large hospital nursery failed to lower mortality or morbidity in comparison with the controls (120).

[4] The first pools were collected by Dr. William Thalhimer in New York. Subsequent pools from plasma collected by the Navy, with the collaboration of Dr. S. O. Levinson in Chicago, were fractionated at the Harvard Pilot Plant and at the Squibb Laboratories under OSRD (CMR) contract with Harvard University.

TABLE VIII

Summary of Results with Normal Serum γ-Globulin
in Common Diseases

Disease	Immunity in Early Infancy	Value of Globulin	
		Prevention	Treatment
Measles	+	+	±
Infectious hepatitis	−	+	o
Mumps	+	o	o
Scarlet fever	+	S	S
Poliomyelitis	+	−	o
German measles	+	S	−
Chicken pox	o	o	−
Infantile diarrhea	o	o *	o
Homologous serum jaundice	−	S	−

Key: + Effective   ± Questionable   o Ineffective
        − Not known   S Under study

* Data from three outbreaks.

Poliomyelitis has been reported by Drs. S. D. Kramer (121) and J. Stokes, Jr., (121) to have been prevented in monkeys, cotton rats, and mice with the Lansing strain, but only when the γ-globulin was injected before, or soon after, the virus. The value of γ-globulin as a prophylactic agent has therefore been doubted by our advisors, since the incidence of the disease in susceptibles is so small that it would be necessary to immunize passively an entire population in order to offer protection to the very small number per thousand who might contract the disease. During the summer of 1944 a carefully controlled study of the therapeutic value of very large doses of γ-globulin in preparalytic cases of poliomyelitis was conducted in western New York State, under the auspices of the American Red Cross, and confirmed the conclusion from previous studies that human antibody does not affect the course of the disease once symptoms have begun (122).

INTRAVENOUS INJECTION OF γ-GLOBULIN

One of the possibilities that arises from the preparation of such a concentrated solution of human antibody is the administration of very much larger doses than could be given as whole plasma. Although intramuscular

injection of doses as large as 100 cc. (the antibody equivalent of 2500 cc. of pooled plasma) has been made, intravenous injection would make possible even larger doses with much less discomfort and a much more rapid establishment of high blood levels. For this reason, the development of preparations safe for intravenous use has been a constant concern. No serious untoward reactions have been observed following several thousand intramuscular injections. Intravenous injection, on the other hand, has frequently produced immediate vasomotor reaction, followed by chills and fever, and although work to eliminate such reactions continues, it has not yet been successfully concluded (123, 124).

Enzymatic Digestion of γ-Globulin

Studies of the splitting of human γ-globulin by various enzymes were undertaken by Drs. Bridgman, Deutsch, and Petermann under Dr. Williams' direction at the University of Wisconsin in the interest of producing antibodies of smaller molecular size. Later these methods were turned toward increasing the yield of γ-globulin and diminishing its depressor effect (125, 126, 127).[5] The course of the enzyme digestion was followed by ultracentrifugal studies, and the products obtained were studied by chemical and immunological methods.

The ultracentrifugal studies[6] were of much value in determining the proper conditions for the digestion procedure. Immunological studies of the pepsin-digested products indicated little or no decrease in the titer of the antibodies usually studied and clinical tests proved their potency against measles. Traces of pepsin were detected in these preparations by the guinea pig anaphylaxis test, but no evidence of sensitization was observed in humans.[7] Digestion by bromelin or papain led to a higher proportion of quarter molecules and to perhaps some destruction of certain of the antibodies. No appreciable destruction of the diphtheria antitoxin activity had been noted,

[5] Part of this work was done under contract with the Committee on Medical Research, and part was carried forth as a part of the program of study of the University of Wisconsin. The immunological studies were conducted at the Department of Bacteriology and Immunology, Harvard Medical School.

[6] These studies indicated that the first step in the digestion of γ-globulin seems to be the disappearance of components of sedimentation constant larger than 7, followed by the conversion of considerable amounts of the $S = 7$ component to slower sedimenting components ($S = 5$ and 3.5) probably representing one-fourth and one-half of the original molecule of $S = 7$. Further digestion results in the formation of considerable amounts of smaller products, and fairly large amounts of nonprotein nitrogen as estimated with trichloracetic acid precipitation tests. The various enzymes used for this digestion affect the course of the digestion greatly. Pepsin gives a large proportion of molecules with $S = 5$, whereas papain and bromelin yield a higher proportion of molecules with $S = 3.5$.

[7] The suggestion of Oncley and Richert that γ-globulin may be digested by plasmin under appropriate conditions and that this procedure would eliminate the use of a nonhuman protein remains to be explored.

however, and the deviations in the other tests were not much greater than might have been caused by experimental error in the limited series carried out. An inability of digested γ-globulin to fix complement was observed and is of interest.

These investigations were urged in the hope that antibodies of smaller molecular size, whether derived from normal or from convalescent plasma, might penetrate more rapidly and therefore be more effective in certain diseases. Although clinical and animal studies on the rate of absorption and on the distribution within the body of these enzyme-digested globulins have been started, they are not completed. It is to be hoped that they can be continued until a clearer understanding of the relationship between molecular properties and physiological behavior is achieved.

VII. BLOOD–TYPING GLOBULINS

Isoagglutinins in Fraction II + III

The anti-A and anti-B isoagglutinins of the plasma were identified in Fraction II + III by Dr. W. C. Boyd, and various more active subfractions were prepared by Mr. Melin in the spring of 1942. Their possible usefulness as reagents for blood grouping was suggested. At that time, the armed forces were using reagents procured from specially selected highly reactive human donors, or produced by artificial immunization of animals, and there appeared to be no interest in the development of processes for preparing isoagglutinins by fractionation of Red Cross blood. Later that year, however, investigations under Colonel G. R. Callender (M.C.), A.U.S., were begun at the Army Medical School, Washington, D. C., resulting in a method of preparing the anti-A reagent by fractionation of human plasma from random donors of group B, and the anti-B reagent similarly from group A plasma (128). Subsequently, Colonel Callender requested that the research on isoagglutinin production be carried out at the Department of Physical Chemistry of the Harvard Medical School, and Lieutenants L. Pillemer and J. Elliott and Sergeant M. C. Hutchinson, from the Army Medical School, were sent to Boston to integrate their studies with the other work on human plasma fractionation.

Plasma from donors of the desired groups, either A or B, typed at the Boston Blood Donor Center of the American Red Cross, was pooled. The isoagglutinins were concentrated in Fraction II + III, and a new procedure (Globulin Method 6) was devised early in 1943, and immediately applied on a large scale in the Harvard Pilot Plant and in a few experimental runs at commercial laboratories. The products so prepared (30) were tested and were generally found to be satisfactory by a panel of consultants set up by the Subcommittee on Blood Substitutes of the National Research Council,

who recommended on July 28, 1943, that these reagents would be useful to the armed forces (31).[1]

On May 17, 1944, the Navy negotiated the first contract for commercial production of isoagglutinin reagents by fractionation of human plasma. Soon thereafter a new fractionation procedure (Globulin Method 7) devised by Dr. J. L. Oncley and Mr. J. W. Cameron, and offering certain advantages in ease of processing, was adopted. Troubles that were encountered in producing materials equal to the Reference Standards could not, however, be eliminated by fractionation improvements alone.

Investigation of the isoagglutinins in plasma itself was undertaken by Mr. M. Melin. The particular difficulty that had to be solved was the poor reactivity of the anti-A reagent (obtained from group B plasma) against cells of the more weakly reacting subgroups of the blood groups A or AB. It was found that random pools of group O plasma yielded a better source of anti-A agglutinin than group B plasma, and that the anti-B activity of group O plasma could be removed by mixing group O and group B blood. Since O donors are encountered much more frequently than B donors, this method makes far greater amounts of blood-grouping globulins available. Mr. Melin, working under our OSRD(CMR) contract, applied these findings to large-scale production at the Fort Worth, Texas, plasma fractionation laboratory of Armour and Company, in September 1944 (42).

Later studies have been directed toward further improvement of the fractionation process. The procedures mentioned so far have sacrificed the other constituents of plasma present in Fraction II + III in favor of the isoagglutinins. Globulin Method 9, described in the earlier pages of this report, makes it possible to save essentially all other components.

Anti-Rh Antibodies

Besides the naturally occurring isoagglutinins, other isoagglutinins may on some occasions arise through the immunization of human subjects. These antibodies include the isoimmune anti-A and anti-B agglutinins, produced in response to injection, and the class known as the anti-Rh antibodies, sometimes produced after injection or sometimes arising during pregnancy, and directed against the Rh factors, which are present in most human red cells, independent of the A and B factors heretofore considered. The concentration and processing of anti-Rh reagents was particularly necessary because of the increasing importance of Rh typing in the prevention of obstetrical

[1] During the evaluation of these products it became evident that the investigators differed widely in the specific numerical values they assigned to the "titer" (referring to the highest dilution at which the material was still found to be active) or to the "avidity" (referring to the speed and extent of agglutination) of a given preparation. Accordingly, two preparations, one of each group, were designated Reference Standards, and later evaluations used these materials as standards of comparison.

accidents as well as hemolytic transfusion reactions. It is now generally accepted that Rh typing should be as regular and routine a procedure as A and B grouping. Since the incidence of antibodies against the Rh factors in serums of human beings is exceedingly low, and these antibodies vary in specificity and are often low in titer, it became important to try to collect large amounts of serum containing anti-Rh agglutinins, pool these, and concentrate and process a useful anti-Rh reagent. Under a separate OSRD (CMR) contract, pools of such serum and plasma were collected under the direction of Dr. L. K. Diamond at the Blood Grouping Laboratory at the Children's Hospital, Boston.

The method for the concentration in Fraction III–1 of the anti-Rh antibodies was in most respects similar to that used for the anti-A and anti-B isoagglutinins (129). However, there were additional difficulties resulting from the specificities of the serums, the greater chemical and physical instability of the anti-Rh agglutinins, the neutralization of the naturally occurring anti-A and anti-B isoagglutinins, and finally the production of a stable, bacteria-free, specific anti-Rh reagent. These difficulties were overcome by using serum albumin as a diluent (45, 130) and it is now possible to produce useful anti-Rh reagents in larger quantity and of much better quality than were heretofore available.

VIII. SUBSTANCES CONCERNED IN BLOOD CLOTTING

The great potential value for clinical use, in many conditions, of prothrombin (131, 132), thrombin (133), fibrinogen, and other components of the clotting mechanism was recognized at the very beginning of our fractionation of human plasma (32) (see pages 371–374). Their separation, purification, and preservation, as well as that of the so-called fibrinolytic enzyme, plasminogen, and of the antihemophilic globulin (134, 135), was complicated by the relative instability of these components of plasma. Work on the substances involved in blood clotting was under the direction of Dr. J. T. Edsall, while the development of fibrinogen plastics and fibrin films was the contribution of Dr. J. D. Ferry.

FIBRINOGEN

Fibrinogen, present in human plasma at a concentration of approximately 2.5 gm./l., clots readily and is much more susceptible to denaturation than most blood proteins. Most of the fibrinogen of plasma was concentrated in Fraction I; the remainder separated in Fraction II + III and, in Method 9, with the prothrombin in Fraction III–2. When care was taken to cool the blood and to mix it well with citrate at the start, as was demonstrated by Dr. R. M. Ferry in 1942, satisfactory preparations of fibrinogen were rou-

tinely obtained from Fraction I. These were preserved in the dried state and readily dissolved on addition of water to form stable solutions. Fibrinogen can readily be further purified, preparations having been obtained in which over 98 per cent of the protein was clottable. The degree of purification and the concentration at which it is dissolved and made available may thus be determined by the uses to which it is to be put.

PROTHROMBIN AND THROMBIN

Prothrombin is far more difficult to separate in pure form. It is present in but small amounts [1] and is quite unstable, both in plasma and in plasma fractions. Conditions for the separation and preservation of human prothrombin as a plasma fraction of great value continue to be sought, but prothrombin safe for clinical use has thus far not been available. The prothrombin concentrated in Fraction III–2 has therefore been promptly converted to thrombin by the addition of calcium ion and tissue thromboplastin. It was considered of importance that no protein of nonhuman origin be introduced into our preparations. As a human thromboplastin, the tissue globulin from placental extract was found to be highly satisfactory (137). The thrombin so prepared could be further subfractionated or could be filtered and dried directly from the frozen state. The latter procedure gave the maximum total yield and provided human thrombin of adequate potency and purity for clinical purposes. Although these thrombin preparations were relatively unstable in solution, losing most of their activity within two or three days at room temperature, they proved extremely stable in dried form. Such preparations have remained essentially unchanged in thrombic activity even after heating at 50° C. for two years.

ANTITHROMBIN

The activity of thrombin rapidly declines in the presence of whole fresh plasma and of certain plasma fractions. Antithrombic activity appeared to be concentrated most strongly in the lipoprotein in Fractions III–0 and IV–1. Fraction III–2, although largely β-globulin (20), is relatively free of β-lipoproteins.

PLASMIN

The dissolution of the fibrin clot, which is so often observed, even under sterile conditions, is due to a proteolytic enzyme in plasma, recently denoted as plasmin (138).[2] In normal plasma the enzyme is present as the inactive

[1] Estimated to be 0.2 gm./l. in beef blood (136).
[2] This enzyme has also been called serum tryptase, for instance by J. H. Ferguson (139) and others.

precursor, plasminogen, which was precipitated in Fraction III–2 together with prothrombin. Plasminogen can be rapidly activated by streptokinase (streptococcal fibrinolysin). It was spontaneously activated, although much more slowly, when the separated fraction stood in the cold for a considerable period of time. In order to permit this activation to proceed it was found important that the lipoprotein fraction, III–0, be separated.

Considerable concentration of plasmin has been effected by Richert, utilizing the ready adsorption of plasminogen by fibrin in order to separate it from the other components of Fraction III–2. The fibrin used for adsorption of plasminogen was subsequently liquefied by the action of the enzyme itself. These enzyme preparations readily passed through sterilizing filters, and the solutions were dried from the frozen state and preserved in active form. Preliminary studies of enzyme activity have been made on this subfraction. The many conceivable clinical uses of the enzyme remain to be explored.[3]

Structure of the Fibrin Clot

The mechanical and chemical properties of the fibrin clots were subjected to profound modification by variations in pH, ionic strength, temperature, and thrombin and fibrinogen concentration, and by the addition of various chemicals such as glycerol to the clotting mixture (33, 140). One of two extreme types of clot prepared by Dr. J. D. Ferry and his co-workers was transparent, gelatinous, friable, and crumbly, with very low tensile strength and elongation and no permanent set; the other was opaque, doughy, and nonfriable and synerized easily with extrusion of water and enormous decrease of volume, yielding a clot of high tensile strength and elongation and high permanent set. The clear type of clot was formed at pH values alkaline to 7, and the opaque type near 6.3.

Both types of clot consist of networks of fibers. In the clear clot the fibers consist of individual chain molecules, whereas in the opaque type they are bundles of molecules aggregated side by side.[4] These studies of the properties of fibrin clots determined the optimum conditions for preparing such substances of clinical importance as fibrin foam and fibrin film.

[3] Other preparations of this enzyme have been obtained, by somewhat different methods, in the laboratory of Dr. J. M. Luck, at Stanford University.

[4] Electron microscopic studies on dried clots were carried out for us, by Dr. James Hillier of the Radio Corporation of America Laboratories, in order to throw further light on the structure of clots. Later, after this chapter was written, a technic was developed, by Dr. C. v. Z. Hawn as a Junior Fellow in Harvard College and Dr. K. A. Porter at the Rockefeller Institute, which allowed the preparation of clots from purified fibrinogen and thrombin (of bovine origin) in a state more suitable for study by means of the electron microscope.

All clots examined were composed of meshworks of single and compound fibers. Initial experiments clearly demonstrated the tendency for the lateral association of unit fibers into compound fibers at the more acid reactions. A striking feature of all the clots studied was the cross-striated appearance of the unit fibers formed. The periodicity of these striae was quite constant and approximately 250Å.

Fibrin Foam and Thrombin in Hemostasis

In February 1942, when active and sterile preparations of human thrombin became available, Dr. I. S. Ravdin at the University of Pennsylvania reported definite benefit from thrombin in controlling bleeding of small vessels, although it was inadequate for the control of bleeding from larger vessels, since the clot formed by the thrombin was rapidly swept away by the further flow of blood. Later in 1942, Dr. Owen H. Wangensteen of the University of Minnesota carried out more extensive clinical studies, with similar conclusions.

In 1943, Dr. Tracy J. Putnam (141) and Dr. Virginia K. Frantz (142) at Columbia University reported using oxidized cellulose in conjunction with thrombin for the control of bleeding. The cellulose provided a solid matrix for clot formation. The possibility of making a similar solid matrix from one of the constituents of human plasma occurred to us. This would have the advantage that the product would not be in any sense a foreign body, but actually a protein of human origin.[5] A fibrin clot of a spongy or foamy texture was prepared by Lieutenant E. A. Bering, Jr., while assigned to the Harvard Plasma Fractionation Laboratory (37). This foamy mass was so strong and rigid that it could readily be cut into slices of convenient size, which were frozen and dried in vacuum. The resulting dried product was then sterilized by heating for about three hours at 170° C.

Before clinical trial was undertaken, experimental studies on the implantation of foam into the brain and other tissues of monkeys were carried out by Drs. O. T. Bailey and F. D. Ingraham (38, 143, 144). Prolonged and systematic observations indicated no disturbing reaction in the site of implantation, beyond that which would have resulted from the formation and subsequent dissolution of a fibrin clot in the same area. After these studies on monkeys had indicated the safety of the use of these products, clinical trial was begun by Dr. Ingraham at the Children's Hospital in Boston and by Major R. G. Spurling at the Walter Reed Hospital in Washington.

The use of fibrin foam and thrombin produced prompt and effective hemostasis in many conditions. It was reported that:

Its most obvious sphere of usefulness is that of the control of oozing from small vessels. The use of foam has materially shortened many neurosurgical operations because the bleeding from the dura, from beneath the edges of the bone flap and from the spinal veins can be quickly brought under control. More important, however, is the fact that fibrin foam adequately controls the oozing

[5] Harvey Cushing over thirty years ago suggested the use of fibrin for hemostasis, and preliminary experiments were made in this direction during the last war. Cushing was, of course, responsible for the widespread use of small pieces of muscle as hemostatic agents, and this proved by far the most effective general technic for hemostasis in neurosurgery up to the beginning of 1943.

from the beds of tumors. This permits the removal of neoplasms *en bloc* which might have had to be removed piecemeal or at several operations if this material were not available.

Another field in which the fibrin foams have proved of great value is in the control of bleeding from large venous channels. When there is oozing from large superficial cerebral veins, especially those entering the mid-portion of the superior longitudinal sinus, ligation is apt to be followed by cerebral softening. Such oozing may be controlled by the use of foam. It is also possible to stop bleeding from the superior longitudinal sinus or other dural sinuses by the use of a good-sized piece of foam placed firmly against the sinus. The foam has also proved invaluable in dealing with blood vessel malformations of the cerebrum, lesions in which the bleeding is well known to be difficult to control by conventional measures. In these situations, the fibrin foam is not merely an agent to shorten neurosurgical procedures, but has succeeded where other agents have failed. Fibrin foam can hardly be expected to control bleeding from large arteries, which is best managed by silver clips. (144)

Production of foam on a moderately large scale for clinical trial was begun under OSRD(CMR) subcontract between the Upjohn Laboratories and Harvard University in January 1944. The resulting materials were distributed for further testing and appraisal to a large group of neurosurgeons. The reports received were so uniformly favorable that the Subcommittee on Neurosurgery of the National Research Council recommended fibrin foam and thrombin for use as a hemostatic on March 28, 1944. Navy contracts for production were made on June 30, 1944. In order to provide for effective control of the product in the clinic as well as in the laboratory, the co-operation of a group of distinguished neurosurgeons [6] was enlisted to test each lot in the operating room before release by the Plasma Fractionation Control Laboratory for use by the armed forces.

Clinical studies of great value were carried on in England, where some of the early material prepared under the OSRD(CMR) contracts had been sent for appraisal to Brigadier Hugh Cairns at the Hospital for Head Injuries at Oxford. On April 18, 1944, Brigadier Cairns wrote to Dr. D. P. Cuthbertson of the Medical Research Council stating: "The Harvard fibrin foam is excellent as a haemostatic agent in stopping bleeding in neurosurgical operations. I have not tried it for arterial bleeding, but for large and small veins and for large venous sinuses like the sagittal sinus I think it is better than muscle. . . . We could usefully distribute 1,000 bottles (with a similar number of bottles of thrombin) at once. The highest priority should be given to the Mobile Neurosurgical Units with our armies in the field." Shipments of a few hundred packages prepared under OSRD(CMR) contract during

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[6] This group has included Drs. J. Browder, P. C. Bucy, C. C. Coleman, W. E. Dandy, L. M. Davidoff, J. P. Evans, W. B. Hamby, G. Horrax, F. D. Ingraham, W. J. Mixter, H. C. Naffziger, E. Oldberg, C. Pilcher, M. M. Peet, B. S. Ray, E. M. Sachs, R. G. Spurling, B. Woodhall, and W. P. Van Wagenen.

the following months reached England for use during the summer campaign of 1944. Neurosurgeons working under the most difficult conditions near the front and behind the lines returned to us a remarkable number of careful and accurate reports on the results thus obtained.

Further studies indicated that fibrin foam and thrombin were of value also in general surgery (58). They have been reported to be of particular value in controlling hemorrhage from the cut surface of the liver and kidney, in prostatectomies (145), gynecologic and thoracic operations, tooth extractions (146), and thyroidectomies, and in stopping the bleeding in hemophilia. Owing to the admirable co-operation of a great number of surgeons, the Plasma Fractionation Laboratory has received a very extensive set of reports on the use of fibrin foam and thrombin in a variety of conditions, which can be generally classified as follows:

	Number of Reports
Neurosurgery	3735
Dental surgery	55
In hemophilia	11
General surgery, miscellaneous *	66
Nose and throat	27
Thyroidectomies	11
Thoracic	38
Bones and joints	15
Stomach, pylorus, duodenum	15
Gall bladder, liver	65
Genito-urinary	54
Gynecological	47
	4139

* Including appendectomy, mastectomy, carcinoma of the rectum, burns, herniorrhaphy, perineorrhaphy, arterial ligation, and hemophilia.

FIBRINOGEN AND THROMBIN IN COAGULUM PYELOLITHOTOMY

The use of fibrinogen and thrombin in the removal of renal calculi had been begun by Dees (147, 148) before our preparations of fibrinogen were available. Subsequently fibrinogen, separated in Fraction I, was employed by Dees, who mixed a solution of these two substances rapidly and injected it into the kidney pelvis, where it was allowed to clot (35) under conditions of a nature to yield the desired mechanical properties (33). After a suitable interval the clot was withdrawn, carrying most of the kidney stones with it. "The use of such a coagulum has the following advantages: (1) All free stones, regardless of size, number, or position within the renal pelvis, should be removed; (2) fragmentation of calculi during removal is avoided; (3) trauma to the kidney is reduced to a minimum; (4) complete surgical

mobilization of the kidney may be unnecessary, as exposure of the renal pelvis alone provides adequate exposure for the procedure" (35, page 576).

Fibrinogen and thrombin have now been used for the pyelolithotomy operation by a large number of other surgeons, and the reported results are closely in accord with those given above by Dees.

Fibrinogen and Thrombin in Skin Grafting

Cronkite, Lozner, and Deaver (149) used fibrinogen and thrombin to produce adhesion between the graft and the underlying tissue, with results that they considered encouraging. Such grafts generally took well and gave results comparable to those obtained by other methods. However, the graft — if not sutured — tended to shrink, so that a larger graft was needed to cover a given area than if sutures were applied. Here again the relative value of fibrinogen and thrombin as compared with other methods still requires critical examination.

Fibrinogen and Thrombin in Burns

Early in our work trial was made of a clot of fibrinogen and thrombin as a coating intended to protect the burned area and to permit healing (36). Studies, both on experimental animals and in the clinic, were made, with results that were in many ways encouraging.

Observations on protective films prepared in situ over experimental wounds in animals, and on human burns from fibrinogen, thrombin, and various plasticizing agents, led to the conception that better control of properties and more convenience in application could be attained by preforming films in the laboratory. It was felt that such films, packaged with sterile precautions, might prove a readily transportable, stable surface agent for burns, which at the time of the African campaigns appeared likely to constitute a major problem in military surgery.[7]

Fibrin Film As a Dural Substitute

Neurosurgical uses of fibrin film were also promptly considered. The histologic sequences following implantation over the exposed cortex in animals were immediately investigated by Dr. O. T. Bailey in collaboration with Dr. Wilder Penfield of the Montreal Neurological Institute; for at that time there existed no satisfactory dural substitute, and its achievement seemed of rapidly increasing importance in reconstructive surgery.

[7] The value of films in burns as yet remains to be demonstrated. With decreasing military need, no surgical group undertook to extend the first observations.

Studies on the structure of fibrin clots already described led J. D. Ferry and Morrison to the preparation of a clot in the form of films and capable, according to the method of preparation, of great variation in thickness, tensile strength, resistance to enzymatic digestion, and other properties (150).

Their use in monkeys was studied by Ingraham and Bailey (39, 151), who found that film could be used to replace dura that had been removed, whether or not the underlying tissue had suffered injury. Adhesions between the dura and the underlying tissue were not observed. The film slowly disappeared in the course of several months and was replaced by a neomembrane of fibrous tissue. Clinical trial of film as a dural substitute followed. In an early and apparently hopeless case of lead encephalopathy wide decompressions were made by Dr. Franc Ingraham and film was used to cover the exposed cortex. The results in this and other human patients confirmed the findings in monkeys, indicating lack of any tendency to formation of adhesions, even over periods which in some cases have now extended to three years. Ingraham and Bailey stated: "In our experience so far, fibrin film has proved more satisfactory than any other material tested as a dural substitute. In addition to its safety, fibrin film is well adapted for use in the repair of dural defects because of its translucence, flexibility, ease of handling, and adaptability to any contour" (151, page 684).

The large-scale production of film began under OSRD(CMR) subcontract between the Armour Laboratories and Harvard University in the latter part of 1944. Under this contract films of excellent quality were rapidly produced and difficult problems concerning packaging and sterilization solved in a relatively short period as a result of the close collaboration between Dr. J. D. Ferry and his group in the Plasma Fractionation Laboratory and Drs. Porsche, Kutz, and Lesh in the Armour Laboratories.

Since satisfactory results appeared to be consistently achieved after a long period of use, and since there was no clinical evidence of the occurrence of adhesions, fibrin film was recommended to the armed forces as a dural substitute at a meeting of the Subcommittee on Neurosurgery of the National Research Council on May 17, 1945. Contracts were shortly afterward made for film for the armed forces, and production began in the summer of 1945. Although no films were produced under these contracts to be released for use before the end of the war, their availability in the postwar period will still be of value to the armed forces, since many operations for head injuries are not carried out for many months after the occurrence of the wound.

Other possible clinical uses of fibrin film have been under trial. Highly promising results were obtained by the use of film and clot in animals for

the end-to-end suture of nerves (152), but clinical tests in man have not yet led to very satisfactory results by this method.

Fibrin film prepared in the form of seamless tubes has been used with great success in blood-vessel anastomosis by Dr. O. Swenson. Clinical trial has not yet been begun, but this use of fibrin tubes appears to be one of the most promising yet reported (155).

Fraction I in the Treatment of Hemophilia

Several years before World War II, injection of a euglobulin fraction of normal plasma had been shown to be effective in shortening the clotting time of hemophilic blood (134, 135). Through the co-operation of Dr. F. H. L. Taylor and his associates at the Thorndike Memorial Laboratory of the Boston City Hospital, all plasma fractions were tested for their effect on hemophilic blood. It was soon found that Fraction I and Fraction III–2 contained a factor in high concentration that reduced the coagulation time of hemophilic blood. The concentration was especially high in Fraction I.

Early in 1945 it became possible to supply Fraction I for clinical trial in hemophilia to Dr. George R. Minot and his co-workers at the Boston City Hospital and to Dr. L. K. Diamond at the Children's Hospital in Boston. Dried sterile Fraction I was used, the usual dose being approximately 200 mg. of protein, most of which, of course, was fibrinogen, which had been demonstrated not to be the active component in this condition. Both intramuscular and intravenous injection were employed. The latter gave the most prompt and striking results, and as yet no unfavorable sequelae to intravenous injection have been reported. In most hemophilic patients, the injection of 200 to 400 mg. of Fraction I from an active preparation caused the clotting time to drop nearly to normal within the first half-hour and to remain lowered for a period of four to six hours or more. The clotting time rose again slowly, but reinjection caused it to fall once more (43, 44, 153). The results have been most striking in children.

Several hemophilic patients who required tooth extractions received injections shortly before this operation, which was then carried out without excessive bleeding. Where oozing was noted after the extraction, and it was found that the clotting time had increased, reinjection of Fraction I immediately lowered the clotting time and stopped the oozing.

In several cases where the patient was suffering from internal hemorrhage, a marked arrest of bleeding was reported as a result of the injection of Fraction I and a reduction in clotting time from the usual hemophilic level of over half an hour to a much more nearly normal level.

In addition to these favorable results, however, there have been several cases — specifically 6 in 52 — of well-established hemophilia in which the results of injection of Fraction I were negative, even when the material used

had been proved to be active in other patients. Also, in some patients who had previously responded either to transfusion of blood or to the injection of Fraction I, there were periods during which the clinical response to intravenous injection of blood or the fraction was unsatisfactory. This was particularly true in patients with bleeding from multiple sites, such as the gastrointestinal tract and the kidney. Variability in response from patient to patient and in the same patient from time to time suggested that conditions diagnosed as hemophilia may not always be due to deficiency of the same chemical component.

Fraction I, like most of the other fractions and subfractions that have been developed, must still be considered to consist of a number of components, one of which has a very high temperature coefficient of solubility, whereas the antihemophilic globulin has a higher thermal stability than fibrinogen. The chemical separation of the diverse components of this fraction, however, remains to be accomplished.

IX. LIPOPROTEINS, HORMONES, AND ENZYMES OF PLASMA

Routine analyses of the blood for clinical diagnosis have in the past generally given the levels of albumin and total globulin and sometimes of fibrinogen and prothrombin. The electrophoretic method of Tiselius readily distinguishes albumin and fibrinogen and the three major families of globulins, designated respectively α-, β-, and γ-globulins. Globulins in each family, moving with slightly different mobilities, have, as we have seen, been recognized and designated α_1- and α_2-, β_1- and β_2-globulins. Chemical fractionation has, however, yielded at least four β_1-globulins and at least two α_1-globulins. The β_1-globulins include the carotene-rich euglobulin, which combines with three times its weight of lipid, as well as high-molecular-weight lipid-free β_1-globulins (Table IV), which are concentrated in Fraction III–o. Fraction III also contains β_1-globulins of different molecular properties. Another fraction, IV–7, which contains the iron-binding component of plasma, is largely lipid-free β_1-globulin and is more closely related to the albumins than to other globulins from the point of view of osmotic activity. Electrophoretically indistinguishable, these different β_1-globulins have no other common property. The lipid-binding plasma component is a β_1-euglobulin, the iron-binding β_1-component a pseudoglobulin. They differ in size, in shape, in solubility, in chemical composition, and in physiological function. Methods of analysis must be developed, therefore, that will yield adequate information concerning the distribution, not only in normal but also in pathologic bloods, of all the different components of the plasma. A low or a high level in any one of them may well reflect unbalance and thus have diagnostic value.

TABLE IX

Components of Fractions Derived from Human Plasma

Fraction	Component	Action	Investigator	Institution	Concentration in Final Product Times Plasma
I	Fibrinogen	Clotting			
	Antihemophilic globulin		Taylor, F. H. L. (43) (44) Diamond (153)	Boston City Hospital Children's Hospital (Boston)	15
II	Measles antibody	Immunity	Stokes (28)	Children's Hospital (Philadelphia)	
	Infectious hepatitis antibody	Immunity	Janeway (29) Stokes (112)	Children's Hospital (Philadelphia)	
	Normal streptococcal antibody	Immunity	Wadsworth (27)	New York State Health Department	22
	Convalescent streptococcal antibody	Immunity	Enders (27) Enders (156)	Harvard Medical School Harvard Medical School	70–130 [a]
	Normal mumps antibody	Immunity	Enders (27)	Harvard Medical School	20
	Convalescent mumps antibody	Immunity	Enders (156)	Harvard Medical School	80–160 [a]
	Pertussis	Immunity	Enders (27)	Harvard Medical School	20
	Influenza A antibody	Immunity	Enders (27)	Harvard Medical School	23
	Diphtheria antitoxin	Immunity	Enders (27)	Harvard Medical School	25
	Callicrein inhibitor		Westerfeld (154)	Harvard Medical School	
	Typhoid H agglutinin	Immunity	Enders (27)	Harvard Medical School	19
III-1	Typhoid O agglutinin	Immunity	Enders (27)	Harvard Medical School	20
	Isoagglutinins		Pillemer (30)	Harvard Medical School	20
III-2	Prothrombin	Clotting	Edsall (32)	Harvard Medical School	25 [b]
	Complement C'1	Immunity	Pillemer (157)	Harvard Medical School	15

	Plasminogen	Proteolytic	Richert (158)	Harvard Medical School	
III–3					
III–o	Antithrombin	Clot inhibitor	Edsall and Miller (56)	Harvard Medical School	30[c]
	β_1-Lipoprotein	Lipid transport	Oncley (52)	Harvard Medical School	
	Carotenoids		Talbot and Mehl (159)	Harvard Medical School	
	Vitamin A	Vitamin	Hickman (160)	Distillation Products	90
	Estriol	Hormone	Roberts and Szego (161)	Worcester Foundation	
	Biotin		Trager (162)	Rockefeller Institute	
IV–o	α_1-Lipoprotein	Lipid transport	Blanchard and Strong (163)	Harvard Medical School	30[d]
IV–1	Wassermann "Antibody"	False-positive inhibition	Neurath (164)	Duke University	
	Growth-Promoting Substance	Diphtheria growth	Mueller (165)	Harvard Medical School	
	Toxic Inhibitor	Inhibition of menstrual toxin	Smith and Smith (166)	Brookline Free Hospital for Women	
IV–4	Hypertensinogen	Blood-pressure regulation	Dexter and Haynes (167)	Harvard Medical School	
IV–6	Esterase	Ester hydrolyses	Taylor and Brauer (168)	Harvard Medical School	300[e]
IV–7	Metal-Binding Globulin	Iron transport Copper transport	Schade (169)	Overly Research Foundation	80[f]
IV	Alkaline Phosphatase	Enzyme	Taylor, F. H. L. (170)	Boston City Hospital	
	Complement C'_2	Immunity	Pillemer (171)	Harvard Medical School	
	Thyrotropin	Hormone	Hisaw (172)	Harvard University	
V	Albumin	Osmotic Transport			5
VI	Follicle-Stimulating Hormone	Hormone	Hisaw (172)	Harvard University	

(a) Concentration referred to normal human plasma.
(b) Calculated on dry-weight basis. Final product about 20 units per mg.
(c) Concentration of β_1-lipoprotein plasma taken as 2 gm./l.
(d) Concentration α_1-lipoprotein in plasma taken as 2 gm./l.
(e) Calculated for 20% solution.
(f) Calculated for 25% solution.

The separation and preparation of each component, whether distinguishable chemically, physiologically, or immunologically, should make possible the discovery of the function and the uses in therapy of the trace components that have been recognized in one or another of the fractions that have been separated in large amounts and that are now also being made available for clinical trial. Among these components is a serum esterase, which appears to be an α_2-globulin, and the iron- and copper-binding β_1-globulin, which has now been crystallized (see footnote 13, Section II) and is being made available for clinical study in a concentration eighty times that in normal plasma.

Plasma components for which satisfactory tests have been reported are listed in Table IX. Extension of this survey is being continued in the postwar world in the expectation that the transport of small organic molecules, in more or less labile combination with specific proteins and lipoproteins, is a major function of the blood. Thus, specific plasma globulins have been separated that combine reversibly with iron and copper and presumably transport them to the tissues, and that dissolve water-insoluble lipids, such as cholesterol, in large amounts, as well as lipid vitamins, such as vitamin A, and lipid hormones, such as estriol. Globulin enzymes with esterase and proteolytic functions have also been separated and concentrated. In other investigations albumins have been demonstrated to combine selectively with organic metal complexes such as mercurials and the sulfonamide drugs, as well as with water-insoluble organic molecules such as naphthoquinones and long-chain fatty acids. The specific interactions between the small organic molecules and the large protein molecules that occur in the blood and in other tissues render it possible to study the interacting physicochemical forces, as well as to explore these natural physiological mechanisms and to determine the possible value of these natural protein complexes in therapy. Knowledge of these interactions and complexes is necessary to our understanding of the equilibrium state in health and of the disequilibrium that reflects disease.

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BLOOD SUBSTITUTES

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THE BASIS for the use of blood substitutes in the emergency treatment of shock stems from two fundamental physiological concepts: first, that wound shock is the result of rapid diminution in blood volume rather than loss of hemoglobin; and second, that the efficacy of any replacement fluid depends on its molecular properties, which determine the osmotic pressure it exerts and the extent to which it is retained within the blood stream. These concepts underlay the introduction of the colloid, gum acacia, during World War I.

In the preceding chapter, Cohn has given the history of the development of the derivatives of human blood — plasma and serum albumin — that were used as blood substitutes. He has also recounted how the program for the fractionation of human plasma grew naturally out of investigations aimed at preparing a blood substitute from animal plasma. Such a development was a matter of urgent necessity between 1940 and 1943, before it became clear that the Red Cross would be able to collect human blood on a scale hitherto undreamed of or that the commercial laboratories would be able to process it to plasma and albumin in equally unbelievable amounts. Thereafter, the search for a satisfactory blood substitute continued, motivated by a desire to find uses for the great amounts of protein being wasted in the discarded red cells and to develop a cheaper, more readily available, and possibly more effective blood substitute.

As the war progressed it became quite clear that there was no such thing as an ideal blood substitute. Although shock could be successfully combated with plasma or serum albumin, the osmotic effects of which were striking, the administration of these colloidal solutions to the wounded unmasked a deficiency of hemoglobin which necessitated the transfusion of red blood cells for its correction. Consequently, whole blood was used increasingly for the treatment of shock in the later stages of the war when it became available at the front, as methods for its preservation and transportation were perfected.

However, a number of substitutes were prepared and tested. These fall into several general groups: derivatives of human plasma; derivatives of animal plasma, derivatives of human red cells, degradation products of other

natural colloids, and synthetic products. As various substances were proposed, the necessity of standards for their evaluation and control became increasingly evident. On the basis of experience accumulated in studies on bovine and human albumin by those working on plasma fractionation, a memorandum was prepared setting forth the general outlines of study for proposed blood substitutes, which served to guide subsequent investigations.

Because of the dependence of physiological effects on molecular properties, all proposed blood substitutes were studied physicochemically. This was done in the departments of physical chemistry of the Harvard Medical School, the Massachusetts Institute of Technology, and the University of Wisconsin. In addition, as a basis for an understanding of the differences in chemical fine structure responsible for immunologic and nutritional differences between various molecules, amino acid analyses were carried out on some of the blood substitutes studied in the College of Physicians and Surgeons, Columbia University.

Pathological studies were pursued in several laboratories. Some were concerned with the general pathologic changes following the injection of various blood substitutes, particularly those that might be expected to evoke antigenic responses. Other investigators devoted particular attention to pathologic changes in the kidney. Studies on rats and dogs provided information on the relative nutritional value of the various blood substitutes. Thus, a large body of fundamental data was accumulated that provided a solid background for interpretation of clinical studies and animal experiments.

PHYSICOCHEMICAL STUDIES

Physicochemical studies of blood substitutes have had two main objectives: the estimation of the size and shape of molecules of the various colloidal materials proposed for use, and the study of the reproducibility and stability of various preparations of these materials.

Estimates of the molecular size and shape of various plasma proteins have been reported elsewhere.[1] These estimates indicate that all the plasma proteins thus far carefully studied have a minimum molecular dimension of about 35 to 50 Å. Molecules of this size appear not to be lost rapidly from the blood stream and are thus responsible for a sustained effect on blood volume. The plasma protein molecules differ considerably in length, however, values from 150 to 700 Å being estimated. The proteins with longer molecules, which have the greatest effect on the viscosity of solutions, are present in small amounts, whereas serum albumin, which has the smallest molecules and therefore exerts the greatest effect on osmotic pressure, makes up half of the protein concentration.

These estimates of the size and shape of the plasma protein molecules were

[1] Pages 400–402.

in many cases derived from preliminary data, but they give good approximate values that will probably not be grossly changed by further studies. The molecular weights are usually those obtained from measurements of osmotic pressure. The dimensions of the molecules are determined on the assumption that they are ellipsoids of revolution. The equatorial diameter is estimated from ultracentrifuge and viscosity measurements, in some cases supplemented by diffusion and double refraction of flow; the volume is estimated from the density and the molecular weight; the length of the molecule is estimated from the diameter and volume.

Serum albumin and hemoglobin are homogeneous protein molecules that have been well characterized. The best estimates of the molecular size and shape of bovine serum albumin are identical with those for human albumin, and the net charge at physiological pH values and the electrical symmetry are very nearly the same. There are, however, slight differences in chemical fine structure revealed by amino acid analyses, on which observed differences in solubility and in immunological specificity presumably depend.

Hemoglobin has a molecular weight very nearly the same as that of serum albumin. Its iron-containing, oxygen-combining pyrrole groups are readily transformed into a series of modifications, and the molecule itself is capable of division into two molecules of approximately equal size, even without loss of its oxygen-combining capacity. Elimination of hemoglobin is far more rapid than would be expected of a protein the size of serum albumin, and this may be explained by the ability to divide into two smaller molecules.

Globin is derived from hemoglobin by extracting the iron-containing pyrrole groups in acid acetone. The protein precipitate, which is white, may be further treated in a variety of ways. Globin, like the products of the dissociation of hemoglobin into half-molecules with a molecular weight only half that of serum albumin, has a diameter more nearly comparable to the dimensions of the plasma proteins than are those of most other suggested blood substitutes. Globin as usually prepared, however, contains a number of degradation products. A series of physicochemical studies of such solutions have been carried out in order to standardize and improve the preparative methods for this material.

The estimates of the dimensions of various blood substitutes are recorded in Table I. With the exception of albumin, hemoglobin, and possibly globin, solutions of these colloidal materials are heterogeneous and contain molecules of widely varying lengths. The molecular weights and lengths recorded for such substances are "number average" values; that is, they are averaged according to the number of particles present. Some of the molecules are very much lighter and shorter, but most of the weight is in molecules heavier and longer than this number average.

Most so-called blood substitutes that have been suggested have diameters approximately half those of the plasma proteins or less (Table I) and may

TABLE I

Estimates of the Dimensions of Certain Plasma Proteins and Suggested Blood Substitutes *

	Molecular Weight	Dimensions of Ellipsoidal Model		Intrinsic Viscosity
		Length	Diameter	
	M	Å	Å	$H \times 10^2$
Serum albumin (human).....	69,000	150	38	4.2
Serum γ-globulin (human)..	156,000	235	44	6
Fibrinogen (human).........	400,000	700	38	25
Serum albumin (bovine).....	69,000	150	38	4.2
Hemoglobin (human).......	68,000	36†	64†	3.8
Globin (human)............	34,000	118	28	7.0
Gelatin:				
Undegraded..............	46,000	330		47.0
P20 type.................	36,000	260	17	36.0
P180 type...............	19,000	140		18.0
Isinglass (degraded).........	29,000	240	16	24.0
Periston...................	37,000	290	16	24.0
Glutamic acid polypeptide ..	15,000	260	11	24.0
Glucose...................	180	9.5	6.5	
Chloride ion...............	35.5	3.6	3.6	
Sodium ion................	23.0	1.9	1.9	

* See Table IV, Chapter XXVIII, for a more complete list of plasma components.
† From x-ray studies, indicating that the hemoglobin molecule is a platelet having the approximate dimensions 36 x 64 x 48 Å.

be expected to leave the blood stream more readily than the plasma proteins. Glutamic acid peptide synthesized by *Bacillus subtilis* and separated as an interesting linear polymer is estimated to be 11 Å in diameter. Gelatin and isinglass are naturally occurring long, loosely coiled fibrous chains 15 to 17 Å in diameter. Pectin has this structure, with a somewhat smaller diameter, and dextran, widely studied in Sweden, appears to be very similar to gelatin and pectin, with possibly a somewhat larger cross section. Periston, a synthetic polyvinyl-pyrrolidone polymer used as a blood substitute by the Germans, also appears to be a material of this type.

Many observations suggest that molecules with a diameter greater than 30 Å do not under normal conditions pass into the urine, but that those with diameters of 20 Å or less leave the blood stream and are excreted in the

urine; the smaller their diameter, the more rapidly this occurs. Among molecules of the same diameter — although the electrical condition of the molecule and the membrane definitely influences permeability — elimination from the blood stream is generally more rapid the shorter their length.

Attempts to increase the cross section of the gelatin molecule by side-to-side polymerization of chains were made. The decreased osmotic pressure and intrinsic viscosity of materials so treated have indicated that this type of reaction does occur. That this product is even more heterogeneous than degraded gelatin, however, is suggested by studies of these solutions in the ultracentrifuge.

Molecules of elongated form are in shape more like fibrinogen than like serum albumin or serum globulin. Fibrinogen greatly increases the viscosity of solutions and causes pseudoagglutination of red cells. Gelatin, pectin, and indeed gum acacia also have these effects. It was recognized that isosmotic solutions of gelatin and pectin would be too viscous to be of value as blood substitutes. They are, however, readily broken down into shorter rod-shaped particles. These degradation products appear to have approximately the same diameters but varying lengths. The problem was to determine whether a reproducible product could be obtained by carefully controlling the breakdown process in such a way that the particle size would become sufficiently small so that viscosity would be reduced and the osmotic pressure increased without reducing the average particle size so much that the effect on blood volume would not be sustained. In other words, would the product when injected be a substitute for plasma proteins rather than for glucose and sodium chloride?

Solutions of gelatin that have been degraded appear to be as satisfactory as any of the other suggested blood substitutes of this group. Degradation decreases the number of very long rod-shaped particles, which are responsible for much of the viscosity and probably for other undesirable effects, whereas it increases the number of very small particles, which are responsible for most of the osmotic pressure, although not for the colloid osmotic pressure, since they are rapidly lost from the blood stream. The longer rodlike particles may be expected to persist longest in the blood stream, and are presumably responsible for the increased sedimentation rate of the red cells.

Because of the interest in gelatin solutions as blood substitutes, a study was made to evaluate the size distribution in gelatin solutions. It is based on the following simple picture of the nature of such solutions, which gives an approximate explanation of their behavior. Collagen consists of long chains of polypeptide residues. The bonds between these residues are usually hydrolyzed at about the same rate, but there are a very few bonds equally spaced along the chain that hydrolyze very much more rapidly. In the preparation of gelatin, nearly all the more reactive bonds and a small fraction of the less reactive bonds are hydrolyzed. The degradation of gelatin consists largely in

the hydrolysis of the less reactive peptide bonds. One may assume an ideal parent undegraded gelatin molecule, which is the length of chain between two reactive bonds (perhaps 800 Å long, with about 1200 amino acid residues in the chain). Gelatin consists of a mixture of such molecules with
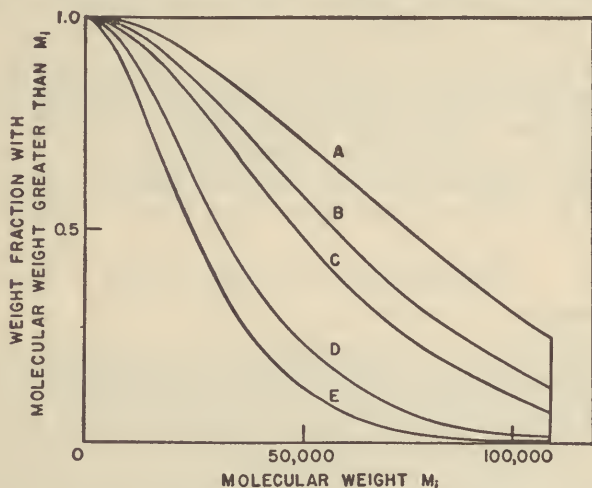
FIGURE 58. *Size distribution for five solutions of gelatin of different degrees of degradation. The number average molecular weights for the solutions follows: A, 45,700; B, 36,000; C, 30,-000; D, 19,000; E, 15,000.*

the products of their degradation, which include every possible peptide from single amino acids to chains containing only one less residue than the parent molecule. A quantitative evaluation of this size distribution has been made for five degrees of degradation of gelatin solutions (Fig. 58). Curve A is that for solutions prepared from high-grade commercial gelatin without additional degradation. Curve B is obtained for slightly degraded solutions, while Curve D is obtained for more degraded solutions.[2] The other curves represent varying degrees of degradation.

PHYSIOLOGICAL AND CLINICAL STUDIES

DERIVATIVES OF HUMAN PLASMA

Of approximately thirteen and a half million bleedings collected by the American Red Cross, over ten million were processed to dried plasma for

[2] Type P20 gelatin solution has been autoclaved at 15 lb./in.[2] pressure for twenty minutes at pH 7.2. Curve D solution has been degraded in the same manner, but with three hours of treatment.

shipment overseas, a relatively small number to liquid plasma for use in this country, and roughly two and a half million to albumin. Most of the investigative work that led to the adoption of plasma by the armed forces was done prior to the war. A few essential studies on the relative merits of plasma and serum, on the comparative stability of various labile plasma components in the frozen, dried, and liquid state, and on the most suitable types of container were undertaken. Since studies on human albumin and other plasma fractionation products have already been described, no further consideration will be given them except to point out that plasma and albumin, both native constituents of normal human blood and satisfying all criteria except that of aiding oxygen transport, were the only blood substitutes actually recommended to or used by the armed forces during World War II.

DERIVATIVES OF ANIMAL PLASMA

Native Proteins

The enormous amount of animal blood that is a byproduct of the meat-packing industry is a vast potential source of blood substitutes. From the physicochemical standpoint bovine serum albumin resembles human serum albumin very closely, and the immediate physiological response to injection of either type of albumin in man is similar. However, immunologic studies show that these two proteins are markedly different. Hence, the study of proteins from animal plasma as blood substitutes was chiefly concerned with the related problems of natural hypersensitiveness (giving rise to immediate anaphylactic reactions) and antigenicity (giving rise to delayed reactions [serum sickness]).

Before the war, Wangensteen and his associates found that injection of bovine plasma into human beings was followed by a high incidence of immediate and delayed reactions. Because of these findings, Cohn was approached and new methods were developed for the separation of bovine plasma into fractions, some of which might be better tolerated.[3] Fraction V of bovine plasma, consisting of approximately 95 per cent albumin and 5 per cent globulin, turned out to be an effective plasma substitute in dogs with experimental burn shock and in human subjects after venesection. Although it could be administered to volunteers without the serious immediate reactions seen with whole bovine plasma, its use was followed by a considerable incidence of serum sickness. Accordingly, in the fall of 1941 clinical tests with this material were abandoned until it could be further purified, on the theory that the serum sickness was due to the globulin impurities.

Such purification was accomplished by the crystallization of bovine serum

[3] Three other groups of investigators (Kremen, Taylor, and Hall; Keys, Taylor, and Savage; and Davis, Eaton, and Williamson) independently demonstrated that the albumin fraction, prepared by various methods, was less likely to evoke reactions than the globulin fraction or whole bovine plasma.

albumin from Fraction V. Clinical investigation was again started with crystallized bovine serum albumin (CBA). Skin tests revealed a very low incidence of sensitivity to CBA as compared to cruder albumin fractions or bovine serum (Table II). The validity of this finding has been borne out by

TABLE II

Results of Skin Tests with Bovine Plasma Proteins

	No. Tested	No. Positive	Per cent Positive
Bovine plasma	153	14	9.15
Bovine fraction V	227	4	1.76
Crystallized bovine serum albumin	2851	10	0.35

the almost complete absence of immediate reactions in a large series of injections of CBA (Table III). As soon as a sufficient number of primary injections had been given without difficulty, the volunteers who had previously had serum disease after an injection of bovine fraction V were given intravenous injections of CBA without reactions, immediate or delayed. This initial exploratory study of the safety of bovine serum albumin in man was carried out in volunteers, most of them students at the Harvard Medical School, whose understanding and interest did much to assist in the early work. After the results appeared to indicate that the injection of crystallized bovine serum albumin was a safe procedure in young men under close observation, the testing program was expanded to include the surgical clinic at the University of Minnesota, where the results were comparable to those obtained in Boston, and the surgical clinic of Johns Hopkins Hospital. Subsequently, the First (Columbia) Research Division of the Goldwater Memorial Hospital, Welfare Island, New York City, joined the studies and made many valuable contributions — chemical, immunological, pathological, physiological, and clinical — to the evaluation of blood substitutes.

Meanwhile, pathological studies in rabbits given large doses of CBA in single and multiple injections showed remarkably few changes. Some of the animals developed precipitins, and with the injection of alum-precipitated CBA in guinea pigs its antigenicity for that species was clearly demonstrated.

By July 1942, injections had been given to nearly 100 human subjects without difficulty, when there occurred the first serious delayed reaction, so severe and atypical that it was only accepted as such when antibodies to CBA appeared in the patient's blood after recovery. This reaction was immediately reported to the Committee on Medical Research and to the National

TABLE III

Comparison of Guinea Pig Anaphylaxis Tests and Human Injections with
Different Types of Preparation from Bovine Plasma

Preparation	Guinea Pig Anaphylaxis			Human Injection*				
	No. In-jected	Per cent Deaths	Per cent Ana-phy-laxis	No. In-jected	Immediate Reactions		Delayed Reactions	
					No.	%	No.	%
Serum	20	75	100	81[a]	—	61 †	42	52
Serum albumin:								
Fraction V				33 ‡	5	15	3	9
Crystallized:								
Least soluble crystals	29	28	69	175	3 §	1.7	18	10
More soluble crystals	19	26	52	110	0	0	9	8
Formaldehyde-treated:								
Serum	20	0	75	100[b]	Few §	—	0	0
Fraction V	22	18	45	8	2	25	0	0
Crystallized Serum albumin	17	6	24	29	0	0	2	7

* First injections only. Patients who died of their disease within a month of injection are excluded.
† This figure was lowered to 24% by absorption with human red cells.
‡ The low percentage of reactions is undoubtedly due to the use of small injections.
§ These reactions were purely pyrogenic, without anaphylactoid symptoms.
[a] Results obtained by Wangensteen before the war.
[b] Results reported by Edwards, from Liverpool.

Research Council. Nevertheless, because of the urgency at that time of the needs of the armed services, the investigators were instructed to continue with the experimental use of crystallized bovine serum albumin in the clinic and to proceed with all possible haste. Crystallization on a larger scale was undertaken, and, after completion of formal undertakings by the proper authorities, plans were made for a large-scale study of the safety of CBA at the State Prison Colony, Norfolk, Massachusetts. Over a third of the prisoners volunteered as subjects after hearing careful explanations of the reasons for the experiment and of the possible risks involved. Of the sixty men who received an injection of one unit each (25 grams of CBA), nearly one-third developed delayed reactions, some mild, some very severe (Fig. 59), and one ending fatally. On September 18, 1942, all clinical testing of these

preparations was stopped, and the Committee on Medical Research was notified that "the kind of reaction observed in these cases appears to be similar to the case of serum sickness previously reported to Washington. . . . They do not resemble previous reactions observed in Boston from amorphous albumin preparations, or those observed by Dr. Wangensteen in Minnesota
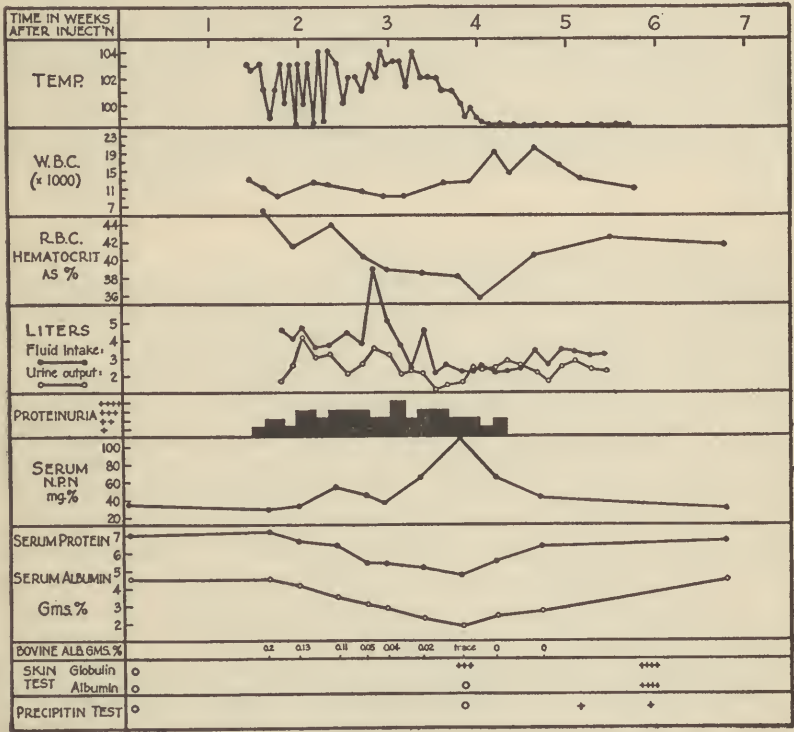
FIGURE 59. *Chart showing the course of a severe delayed reaction to crystallized bovine albumin in a volunteer.*

following the injection of unfractionated bovine serum or plasma. Until our chemical investigations are completed, all clinical trial has been suspended." Nevertheless, all the volunteers indicated their willingness to participate in further clinical studies as soon as the product could be improved to justify the resumption of human tests.

Intensive studies were made to determine the cause for the discrepancy between the results observed in the initial period with material processed on a small scale, with Harvard Medical School students as volunteers, and in the later period with material prepared on a much larger scale and tested

in prisoners and patients with chronic disease. This work has been hampered by the lack of an animal test for antigenicity, which could be correlated with the results in man. Numerous leads have been investigated, particularly the presence of partially denatured protein, contamination with heavy metals, and finally the use of a somewhat different crystalline fraction. However, with all types of preparation tested, serum sickness continued to occur in about 8 per cent of those injected (Table III). Thus, although crystallized bovine albumin is definitely less antigenic in rabbits, guinea pigs, and human beings than bovine plasma and can be injected without fear of immediate reactions in doses of 25 gm., it appears to sensitize about one person out of twelve. In this work, intravenous injection of a dilute solution of procaine hydrochloride was found to relieve the symptoms of serum sickness.

Modifications of Bovine Serum Albumin

Long before the first reactions to CBA were observed, another method of approach — namely, chemical modification of the protein in a less antigenic form — was attempted. A number of investigators tackled this problem. Dr. Beatrice Segal undertook to assay the antigenicity of these modified albumins, while chemical control of the products was Dr. Cohn's responsibility. Of the many different methods tried, none were wholly successful, and the work may be summarized with the following statements: it was possible to alter the immunologic specificity by the addition of new groups, but such altered proteins were antigenic and often more toxic than the native protein; it was possible to abolish antigenicity by a number of chemical methods, but any method so far tried that was successful led to protein denaturation, with alteration in the physicochemical properties on which its function as a blood substitute depends.

Edwards in England showed that by treating bovine plasma with heat (72° C.) in the presence of dilute formalin and ammonia it was possible to destroy its globulins with their reactive properties and to leave a material that appeared to behave like an albumin solution and that did not produce immediate or delayed reactions when given in large doses. The diminished antigenic power of such despeciated bovine serum was confirmed in animals (Table III). Following this lead, Hughes prepared modified CBA by formalin treatment, obtaining a product that appeared to be less antigenic in animals but retained its physicochemical properties. Use of this preparation by Wangensteen resulted in an incidence of serum sickness similar to that with the use of unmodified CBA (Table III), and clinical trials have therefore been abandoned until such time as better methods of modification may be devised.

Before leaving the subject of crystallized bovine serum albumin, it should be pointed out that although it has not yet proved safe as a substitute for

plasma in man, its development has provided investigators with a valuable reagent — a highly purified protein available in large amounts for all types of research.

Derivatives of Human Red Cells

A problem of great concern to those associated with the Red Cross blood program was the waste of human red cells discarded after separation of the plasma. In addition to studies on the preservation and transfusion of resuspended cells,[4] two other lines of investigation were pursued that might have led to their better utilization. One concerned the use of native hemoglobin and the other the development of a hemoglobin derivative, globin, as a blood substitute.

Hemoglobin

Hemoglobin is a normal constituent of human cells but an abnormal constituent of the body fluids, and when it appears in the plasma, as a result of rapid hemolysis, it is partly excreted in the urine as hemoglobin but is mainly converted to iron, bilirubin, and its protein moiety, globin, which can be utilized to form new hemoglobin and plasma protein. In several conditions associated with hemoglobinuria, notably transfusion reactions and blackwater fever, anuria and renal failure are prone to develop, and thus the idea that hemoglobinuria leads to renal damage has become prevalent.

Amberson had demonstrated that solutions of hemoglobin in an appropriate electrolyte diluent could be used both for their oxygen-carrying capacity and for their colloid osmotic pressure to sustain life temporarily in animals from whom over 90 per cent of the circulating blood had been removed. Other investigators had shown that human hemoglobin could be prepared in such a way that injections of moderate doses were well tolerated. In the course of studies on shock, it was reported that dogs suffering from severe hemorrhagic shock could be resuscitated in a remarkable manner by the injection of hemoglobin solutions.

Research workers at the Rockefeller Institute took up the study of hemoglobin in connection with an investigation into the effects of shock on the kidney. In the course of this work several valuable contributions were made, including the copper sulfate method for determining blood and plasma specific gravity and a method for the preparation of relatively pure, stable, sterile oxyhemoglobin. In dogs its half-life after injection was eight hours, approximately 35 per cent being excreted in the urine in twenty-four hours. Transient depressions of urea clearance, such as have been observed in shock alone, but few instances of permanent change in renal function, were observed when dogs with hemorrhagic shock were given large amounts of

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[4] See Chapter XXX.

70 per cent hemoglobin solution. Even infusions of methemoglobin were capable of restoring the flow of urine in shock. Thus, hemoglobin solutions have considerable interest as blood substitutes, both for their colloidal properties and for their oxygen-carrying power. However, amounts of hemoglobin comparable in oxygen capacity to the red cells would have enormous osmotic activity until lost from the blood stream, and the danger of renal damage when hemoglobin is given to a patient in shock remains a definite possibility.

Modified Globin

By removal of the pyrrole groups of hemoglobin with acid acetone, followed by alkali treatment of the remaining globin, a modified globin has been prepared by Strumia and his associates that has a molecule more nearly globular than is the case with any other substitute. The principal difficulty with globin has been the development of methods for obtaining a uniform product and one free of immediate vasomotor reactions, presumably due to the presence of certain large molecular aggregates or degraded protein. Modified globin has an average molecular weight of 34,000 and a molecule about three fourths as long and as thick as that of serum albumin. Thus, a solution with the same osmotic pressure as an albumin solution is considerably less concentrated and slightly less viscous. Approximately 24 gm. of globin can be prepared from the erythrocytes of a single 500-cc. blood donation. This has about three times the osmotic activity of the 17 gm. of plasma protein contained in the same amount of blood.

Clinical and physiological studies with globin are still proceeding. In lots from which reactions have been eliminated, the injection of large or multiple doses has been well tolerated, and sensitization has not developed. Globin has proved effective in raising blood pressure in a small group of cases of surgical shock, and has been shown to increase the total circulating protein in the plasma and to induce a positive nitrogen balance in patients with hypoproteinemia. Studies in dogs demonstrate that human globin can be utilized to form new hemoglobin and plasma protein. Molecules the size of globin have usually been thought of as traversing the capillary wall rather readily. Experiments with rats show that a much greater increase in protein excretion occurs after the injection of globin than after that of human serum albumin; in fact, the excretion rate is very similar to that for Bence-Jones protein, which has similar dimensions. Considerably more evidence is needed on the distribution and fate of this suggested blood substitute in various conditions before its therapeutic value can be assessed.

DEGRADATION PRODUCTS OF OTHER NATURAL COLLOIDS

Nature possesses many examples of large molecules with lengths far greater than their diameters. Two types are readily obtained and have been

tested as blood substitutes: the proteins of connective tissue of animals and fishes and the complex polysaccharides of the vegetable gums.

Proteins from Animal Sources

Gelatin. Degraded gelatin from bones or skins of cows and hogs has been studied as a potential blood substitute. It has the great advantage of being nonantigenic and sterilizable by heat, and the manufacturers have demonstrated that it can be prepared on a large scale free of pyrogenic material. From the military standpoint it has the disadvantage of being in a semi-solid state at ordinary temperatures, so that it must be warmed to 35° C. for use. Pseudoagglutination interferes with the typing of blood from patients who have been receiving gelatin, but may be rectified by the addition of glycine.

The injection of gelatin, once the problem of pyrogenic reactions was solved, has not been attended by untoward effects, except for occasional urticarial reactions and moderate frequency of venous thrombosis. The latter may have been probably due to mercurial preservative in the solution, since after its omission the incidence of thrombosis was diminished. Studies have shown a close correspondence between molecular size and physiological effect. High-viscosity gelatins give more sustained hemodilution and a slower excretion of gelatin in the urine than do those of low viscosity (Fig. 60). The efforts of the chemists to assist in the development of standardized gelatin solutions for clinical trial were valuable, since results differ so markedly with different gelatins. As with gum acacia, gelatin on injection appears to displace some of the normal plasma protein from the circulation (Fig. 61), but unlike the colloids derived from vegetable sources, it does not lead to deposition of material in the liver and spleen. Some changes in the kidney tubules due to the excretion of gelatin in the urine have been observed. It is possible that renal damage may account for the observation of some toxic effects on prolonged administration of gelatin to dogs.

It is apparent from the clinical studies that gelatin can serve a useful purpose in the emergency treatment of shock if blood or blood derivatives are not available. It has been employed in a considerable number of cases of surgical shock with satisfactory results. Its greatest advantages are cheapness, availability, and apparent safety for emergency use. Against this must be balanced the fact that the molecular shape is not ideal for a blood substitute, since the most osmotically active particles are lost most rapidly from the circulation and the larger particles lead to undesirable effects, such as conglutination of the cells and an increase in the viscosity of the blood. Another disadvantage is the fact that gelatin is an incomplete protein and does not contribute appreciably to the formation of new protein except as supplemented by food in the diet. This has been clearly demonstrated by work on rats and is consistent with the knowledge of gelatin derived from a long series of classical studies in nutrition.
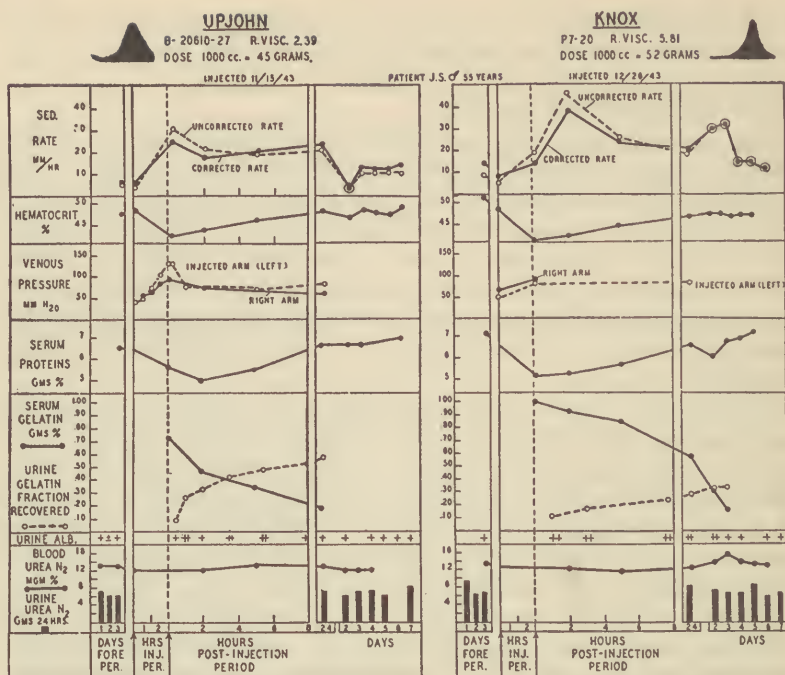
FIGURE 60. *Comparison of effects of injection of two gelatin preparations in the same subject.*

Isinglass. In Canada, Taylor has studied the properties of isinglass, a gelatin derived from the swim bladders of fish. Its principle advantage over animal gelatin is the fact that it is liquid at ordinary temperatures, but since its average molecular size is somewhat smaller, it is lost from the circulation quite rapidly, like markedly degraded gelatin.

Modified Gelatin. Since the asymmetric shape of the gelatin molecule is not ideal, attempts have been made to treat it chemically so as to produce a molecule with a greater diameter, which should have more desirable properties. At the California Institute of Technology modifications of gelatin, globin, and bovine albumin have been developed. The most promising modification involved the treatment of gelatin with glyoxal and oxidation by autoclaving with hydrogen peroxide, causing alterations in both viscosity and colloid osmotic pressure. Oxypolygel, as this material was called, appears to be uniformly nonpyrogenic and retained better in the circulation of animals than ordinary regraded gelatin of comparable molecular weight. Clinical studies have not been reported.
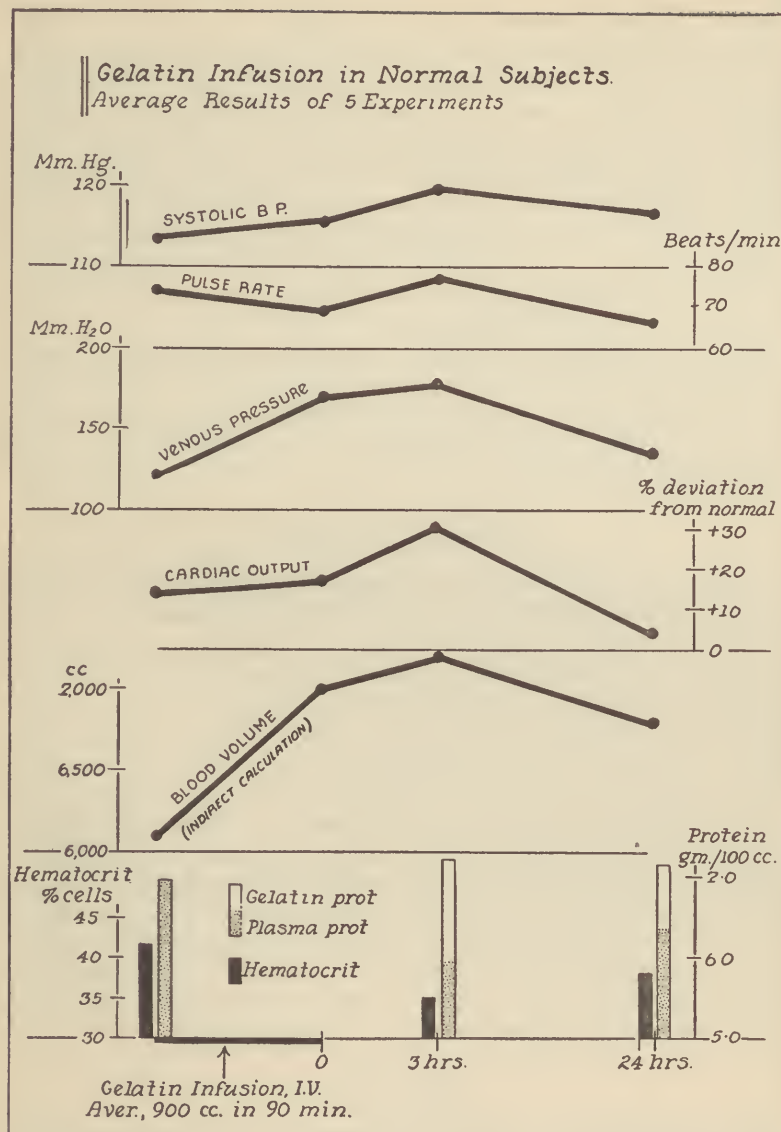
FIGURE 61. *Average results obtained in five normal subjects before and for 24 hours after a large infusion of gelatin intravenously.*

Complex Carbohydrates of Vegetable Origin

Gum Acacia. Gum acacia was not restudied during World War II, because it was believed that the evidence accumulated as a result of its introduction during the previous world war and its subsequent use in the intervening period indicated definitely that it had certain undesirable properties. It is an unnatural type of colloid, following the injection of which normal plasma protein is rapidly displaced from the circulation. Although acacia has been used in clinical medicine as a diuretic agent with considerable success, it is gradually deposited in the cells of the liver. Here the accumulation after repeated injections may impair liver function, particularly the ability of this organ to produce plasma proteins.

Pectin. Pectin has been suggested as a possible blood substitute. Its properties appear to be closely similar to those of gum acacia, and there seems little doubt that although it may be used as well as acacia to maintain the circulation in shock, it suffers from exactly the same drawbacks and is not a suitable substitute for general use.

SYNTHETIC PRODUCTS

Production of a synthetic blood substitute has appealed to many workers. Attempts to modify bovine serum albumin, a natural molecule adapted for its physiological function, in order to render it safe for use represents a partial attempt at synthesis of a suitable substitute. Attempts to change the configuration of gelatin in oxypolygel are based on the same principle as similar attempts to render asymmetric molecules more suitable for a function performed in nature by symmetrical molecules. Four synthetic products have received consideration as blood substitutes during this war. Periston, a polyvinyl pyrrolidone, was captured from the Germans in North Africa, but had limited study in this country. Glutamic acid polypeptide is produced by the growth of a strain of *B. subtilis*. It is nontoxic and nonantigenic in animals but, as might have been expected from its molecular dimensions, is rapidly lost in the urine. Sodium glycerol polysuccinate likewise proved nontoxic but also was rapidly excreted in the urine. Dextran, a polysaccharide produced from sucrose by certain bacteria, has been studied in Sweden, but has not been subjected to physiological study in this country.

SUMMARY

Co-ordinated studies on various types of substances proposed as blood substitutes have demonstrated the complete dependence of physiological behavior on chemical structure. The molecules in nature that normally maintain the volume of circulating plasma are of a diameter above 30 Å and are relatively symmetrical, giving solutions of low viscosity. Animal plasma

proteins duplicate these desirable physicochemical properties but are unsuitable because of their antigenicity. It has not been possible to destroy antigenicity without changing the molecular structure responsible for such properties. Solutions of human hemoglobin provide not only colloid osmotic pressure but also oxygen-carrying power, but hemoglobin and its derivative, globin, tend to split into molecules of smaller size than albumin, with increased excretion in the urine. Hemoglobin can perhaps be prepared in safe form, but its effect on the kidney in the face of markedly diminished renal blood flow calls for further study.

The blood substitutes derived from fibrous proteins of animals or fishes (gelatin and isinglass) and the polysaccharides of vegetable origin (acacia and pectin) all owe their effect on colloid osmotic pressure to the length of the molecule. Although control of particle size is possible through control of the period of degradation by autoclaving, the resulting solutions are not homogeneous and contain short molecules, which are rapidly excreted, and long molecules, which are retained but increase blood viscosity and lead to pseudoagglutination of the cells. Furthermore, these substances, unlike plasma proteins, hemoglobin, and globin, do not contribute to the body protein pool. Gelatin is apparently harmless if properly prepared, and may be used to combat shock quite successfully, but it displaces plasma protein temporarily from the blood, while acacia and pectin not only do this but may be deposited in the liver.

Certain other blood substitutes, derived by chemical or bacterial synthesis, are likewise rapidly excreted, because of the small diameter of their molecules. Polymerization and oxidation of gelatin have produced considerable change in its physical properties leading to altered viscosity and greater retention in the blood.

To date, it has not been possible to find a physiologically ideal substitute for the native plasma proteins. In closing, it should be emphasized that the experience of surgeons everywhere emphasizes the importance of oxygen-carrying power as well as colloid osmotic pressure in adequate replacement therapy of the blood losses sustained as a result of trauma.

CHAPTER XXX

METHODS OF PRESERVATION OF WHOLE BLOOD

GEORGE M. GUEST

As POINTED OUT elsewhere in this history, one of the first tasks undertaken by the Committee on Medical Research was to find practical ways to restore the circulating blood volume in wounded men in order to avoid or combat shock. For this the provision of human blood plasma was regarded as the first and paramount need, and the principal effort was directed to problems of drying and storing plasma and devising improved procedures for its administration. Experience reported from various battle fronts before this country's entry into the war indicated, however, that in some cases of injury with gross hemorrhage whole blood rather than, or in addition to, plasma was required. Various consultants recommended early that steps be taken to provide the armed forces with whole blood drawn from donors in the United States, but this proposal was thought at first to be impractical, largely because of difficulties visualized for the transportation and delivery of blood to distant points for use within the period of time allowed by the existing methods of preservation.

It was also thought at first that whole blood in adequate amounts could be obtained from donors among the military personnel in each area. Blood banks established by various service commands in the Mediterranean, European, and Pacific Theaters did supply large amounts of whole blood that proved invaluable during several major campaigns; but with progress of the war it became increasingly evident that the demand would exceed the possible supply of blood from such sources alone. Moreover, the widespread distribution of malaria and infectious hepatitis in some areas made the selection of service-personnel donors increasingly difficult or even hazardous and the need for whole blood to be supplied from home sources more imperative.

The value of whole blood for transfusion, compared with that of plasma or plasma products, lies mainly in the ability of the red cells to survive and perform their physiological functions (for example, transportation of oxygen) in the circulation of the recipient. Any practical program for the provision of whole blood to battle fronts required that the cells should remain well preserved for a sufficient length of time to allow its shipment over long distances and its storage at the destination for a reasonable period of time

while awaiting emergency use. By the method of preservation generally employed in most hospital blood banks, the red cells can be kept for only five to seven days in a satisfactory condition for transfusion; provision for longer preservation is of course unnecessary when quick use of the blood is anticipated. For overseas shipment of blood the military services stated that a minimum period of twenty-one days of safe preservation would be desirable, perhaps essential.

To meet this requirement, the Committee on Medical Research sponsored an extensive program of research on many problems connected with the preparation, storage, and transportation of whole blood. The program involved the close collaboration of physicians, biochemists, physiologists, hematologists, and physicists, in research teams working in widely separated centers in the United States and Canada, with frequent meetings for discussion and exchange of information under the auspices of the Committee on Transfusions and the Subcommittee on Blood Substitutes, Division of Medical Sciences, National Research Council. Highly successful, their studies brought increased understanding of many factors involved in the deterioration of cells in stored blood. The results led to the definition of conditions found to be essential for satisfactory preservation of whole blood and to the selection of a preservative fluid deemed to be the best of a large number tested. The fluid chosen, known as ACD (acid-citrate-dextrose or Loutit-Mollison's solution), was adopted during the latter part of the war for the preservation of the blood collected by the American Red Cross for the Army and Navy, to be shipped overseas by air transport to both European and Pacific areas. Satisfactory preservation of this blood for at least twenty-one days was assured, and many bloods could be — and were — used after as long as thirty days of storage.

Problems involved in the storage of whole blood for transfusion purposes and existing knowledge on the subject were thoroughly reviewed at a conference of the Subcommittee on Blood Substitutes held in Boston on May 25, 1943. This review emphasized the fact that despite a large background of research already done in this country and abroad, there was still a paucity of exact knowledge of many of the factors involved in the preservation of red cells in stored blood; for example, effects of temperature within varying ranges of refrigeration, degree of dilution and specific effects of the chemical constituents of various diluting fluids that had been recommended, range of pH of the blood mixtures, effects of different salts, and the role of the enzyme systems of the cells in maintaining functional and morphologic integrity of the cells.

A program of research, to be co-ordinated in conferences of this sub-committee, was planned to follow two broad lines; namely, studies of physicochemical changes found in the erythrocytes of stored blood to establish criteria for judging their state of preservation or deterioration in vitro, and

studies to determine the ability of variously preserved cells to survive in vivo, after transfusion, in the circulation of a recipient. The collaborating investigators agreed early that the ultimate choice of any method would be made on the basis of viability of the cells as proved by their post-transfusion survival.

Several groups of investigators adopted different methods of approach to the common problem according to their special backgrounds of experience and the facilities available to each. Under support of the Office of Scientific Research and Development, in vitro studies of physicochemical properties of stored red cells in varying states of preservation or deterioration were carried out at Princeton University, the Cincinnati Children's Hospital Research Foundation, and the Harvard Medical School. Many in vitro studies also were carried out as measures of control by the investigators principally occupied with post-transfusion tests of viability of preserved cells. Studies of in vivo cell survival were carried out in projects at the Harvard Medical School, the Massachusetts Institute of Technology, the Evans Memorial Hospital, Boston, and the Bryn Mawr Hospital. In addition, a number of investigators working independently along closely related lines participated actively in the program by attending blood preservation conferences of the National Research Council and exchanging information of common interest.

For testing the post-transfusion survival of variously preserved bloods two methods have been used to distinguish donor cells from recipient cells: the differential agglutination technic, developed by Ashby in 1919, and the radioactive iron tracer technic, which had its first extensive practical application in the Committee on Medical Research blood preservation research program. The Ashby technic is extremely tedious and in some hands fraught with errors leading to wide variations in results, discouraging to many investigators, but it has distinct advantages for following the survival of cells for long periods of time after transfusion, inasmuch as the identification of the cells depends on unchanging characteristics, the specific blood-group agglutinogens.

The Ashby method involves the transfusion of group O whole blood, the state of preservation of which is to be tested, into group A recipients. At suitable intervals of time after the transfusion, samples of blood are drawn from the recipient, and mixed with anti-A serum to agglutinate the recipient's own cells, and the group O donor cells remaining in suspension are counted. An important contribution from this type of study was evidence that transfused *fresh* donor cells are eliminated from the recipient's circulation at a fairly steady rate, approximately 1 per cent per day: for example, the normal cell elimination curve could be represented by a straight line falling from 100 per cent to zero in one hundred to one hundred and twenty-five days. Presumably the points along this line would represent the maximum life of erythrocytes of varying ages in a given sample of normal blood,

from that of the most mature cells, which disappear first, to that of the youngest cells, which disappear last. This method also gave results confirming conclusions drawn from the isotope tracer experiments (to be cited) to the effect that practically all the *poorly preserved* donor cells in a given sample of stored blood actually disappear from the circulation of the recipient within the first few hours after transfusion.

The radioactive iron isotope method for labeling erythrocytes was based on work started in 1939 at the University of Rochester by Hahn and Ross, who found that radioactive iron (Fe ^{59}) when fed or injected into dogs became incorporated in hemoglobin molecules, thus tagging the red cells. This labeling was found to be specific, in that there was no transfer of iron from the erythrocytes while they remained intact. At Boston University in 1942 this tracer method was first applied in human transfusion experiments.

At about the same time investigators engaged in research on shock under the Committee on Medical Research developed a method for the measurement of circulating red-cell volume by means of two radioactive isotopes of iron, and this double-tracer method was promptly applied in the studies of blood preservation. Compared with the Ashby method, the isotope technic for artificially labeling erythrocytes permits far more exact determination of the number of donor and recipient cells in any mixture of the two, with a great economy of human labor, but it is complicated by the fact that shortly after the labeled donor cells disintegrate in the body of the recipient the liberated tracer iron is reutilized and reappears in the recipient's own newly formed erythrocytes, which are thereafter indistinguishable from the surviving labeled donor cells. Hence, the principal usefulness of the tracer method is found in the first few days after transfusion; namely, in demonstrating the rapid disappearance of poorly preserved donor cells from the recipient's circulation.

The investigators engaged in the in vivo cell-survival studies were supplied with radioactive iron isotopes prepared in the cyclotron of the Department of Physics, Massachusetts Institute of Technology. The two isotopes employed were Fe^{59}, with a half-life of forty-seven days, emitting low-energy beta rays, and Fe^{55}, with a half-life of about five years, emitting low-energy x-rays. These isotopes were obtained by bombardment of cobalt and of manganese, respectively. Fe^{59} had been used in red blood cell studies since 1939, but Fe^{55} was apparently first used for biologic research in this wartime program. Each isotope was prepared, mixed with ordinary iron, in the form of salts suitable for administration to prospective blood-donor subjects by mouth or by injection. The radioactivity center at the Massachusetts Institute of Technology also undertook the analyses of radioactivity in the post-transfusion blood samples drawn, in the widely separated research centers, from the recipients of isotope-labeled blood the state of preservation of which was being tested. To expedite this work special methods were developed

for handling the analyses in large series, employing special types of Geiger-Müller counters for distinguishing two isotopes in the same sample and ingenious devices for automatic sample-changing and automatic recording of the radioactivity data.

The radioactive iron was administered in small doses to volunteer prospective donors (usually medical students) for two to four weeks, after which time the isotopes were found incorporated in the hemoglobin of the circulating erythrocytes in amounts adequate for detection. Thus assimilated, the radioactive iron atoms behave like all other normal iron atoms in the body, being chemically indistinguishable from them and accompanying the normal iron atoms in an unchanging proportion throughout all their metabolic transformations, thus acting as tracers or spies, which by their presence disclose in detail the movements of the normal atoms that they accompany. It is estimated that in the tagged erythrocyte there are only one to ten radioactive iron atoms to about a thousand million atoms of ordinary stable iron; yet the sensitivity of the methods of measurement is such that these atoms can be detected with extraordinary accuracy.

Blood containing the labeled erythrocytes was drawn from the donors into various fluids the effectiveness of which for cell preservation was to be tested. The mixture of blood and fluid was distributed into several flasks and stored for different periods of time at 4° C., or for similar periods at different temperatures, after which the samples were transfused into volunteer recipients. In final tests some of the flasks were shipped by air, with or without refrigeration, to distant points and then returned to the research centers, where they were stored at 4° C. for varying periods of time before transfusion — thus duplicating as far as possible the conditions encountered in the military services. At suitable intervals during storage of the blood and at the time the transfusions were performed, small aliquots of each sample were taken for various in vitro tests so that the in vitro criteria of preservation might be correlated with observations on the in vivo viability of the cells.

Following each transfusion small samples of the recipient's blood were drawn at appropriate intervals, ranging from twenty minutes to ninety-six hours, to determine the number of surviving cells by measurement of their radioactivity. The volume of cells and plasma in the recipient's circulation was determined before transfusion as a basis for calculating the expected initial post-transfusion radioactivity of the blood that would correspond to 100 per cent retention of the transfused cells. Diminishing radioactivity in successive samples of the recipient's blood drawn after transfusion furnished a measure of the rate of removal of the donor cells from the circulation. In the case of partially deteriorated bloods this rate was at first rapid, then slower, the data indicating that the majority of the nonviable erythrocytes actually disappeared from the recipient's blood stream within the first

three to eight hours after transfusion. Usually the lowest values for radio-activity were found at around twenty-four to forty-eight hours, after which time a rise in activity indicated a reutilization of the tracer iron liberated from destroyed labeled erythrocytes.

Having agreed that the survival of transfused cells is the ultimate test of their state of preservation, the Subcommittee on Blood Substitutes adopted a rule to the effect that the retention of 70 per cent of the transfused cells for forty-eight hours would be accepted as indicating a satisfactory state of preservation, and 90 per cent retention or more as indicating optimal preservation.

In vitro studies were undertaken under the general proposition that the red cell is a functional unit in which all physical and chemical properties are interrelated, and that the integrity of the cell is dependent on its meta-bolic activities as well as on its physical structure. With this concept in mind, the investigators carried out a wide variety of tests on samples of blood that were mixed with various proposed preservative fluids and stored for varying periods of time at varying temperatures — tests designed to yield a broad description of the changing chemical and physical properties of the cells in stored blood at different stages of preservation and deteriora-tion.

Briefly summarized, the most important of the cellular changes found to be associated with processes of deterioration were the following: increasing osmotic fragility, as indicated by hemolysis in hypotonic salt solutions; dimensional changes, crenation, decreasing diameter, and increasing thick-ness; decreasing concentration of adenosine triphosphate and to some extent of other fractions of the total organic acid-soluble phosphorus, with increased concentration of inorganic phosphorus; decreasing concentration of potas-sium, with its escape into the plasma; loss of glycolyzing power; and de-creased ability to utilize oxygen. In general, critical degrees of change in each of these characteristics appeared to be interrelated and closely asso-ciated with stages of preservation (reached early or late, according to the conditions of storage), which were at the same time shown to be suboptimal or unsatisfactory by parallel in vivo tests of post-transfusion survival. Taken singly, the osmotic fragility of the cells was more closely correlated with post-transfusion cell survival than any of the other indices, but all the indices mentioned had to be considered in order to gain the fullest information, from the in vitro studies, on the effectiveness of the various preservative methods.

An especially noteworthy conclusion drawn from these studies was that mere absence of spontaneous hemolysis in stored blood (evidenced by the appearance of free hemoglobin in the plasma) could be a misleading index of the state of preservation of the cells. When hemolysis of some cells in a given sample has occurred, it does generally mean that the other cells in the sample are in late stages of deterioration, and it is proper that the blood

should be discarded. It was found, however, that the absence of visually perceptible hemolysis was not correlated with other in vitro indices of preservation or with the viability of the cells following transfusion. In some bloods showing no significant hemolysis, gross deterioration of the cells was shown by the fact that their post-transfusion survival in the circulation of a recipient was almost zero at the end of twenty-four hours; this was especially true in the case of some bloods that had not been properly refrigerated.

The optimal range of temperature for the preservation of whole blood, in all the preservative fluids tested, was found to lie between 4 and 10° C. Exposures to temperatures outside this range led to progressive deterioration of the red cells, and the rate of deterioration was markedly accelerated by temperatures above 15° C. Moreover, the deterioration initiated by short periods of exposure to higher temperatures was not notably retarded by subsequent storage of the blood at the optimal temperature.

A large number of preservative fluids proposed in the past, as well as a large number of modifications of these fluids suggested during the research program, were each studied more or less completely by the methods just reviewed. Some of the principal fluids thus tested were 4 per cent solution of trisodium citrate, the diluent employed most commonly in hospital blood banks in the United States; citrate-dextrose, or Whitby's solution, as employed by the Army Transfusion Service of Great Britain; DeGowin's solution; Denstedt's or McGill's solution; Alsever's solution; Muether's solution; and Loutit-Mollison's solution. The last-named solution was first selected on the basis of in vitro screening tests as the most promising type of preservative, but a great deal of work on many modifications of the general formula was carried out before general agreement among the various investigators was reached and a definitive recommendation was made.

The essential characteristics of these various fluids may be mentioned briefly. In addition to cell-preservative qualities, the first essential, certain practical considerations affected the ultimate choice. The dilution factor for the different fluids varies widely, being 1:10, parts of fluid to parts of blood, in case of the simple citrate solution, 1:3 for Whitby's solution, 1:1 for Alsever's solution, and 1.5:1 for DeGowin's solution, thus requiring between 50 and 750 cc. of fluid to be added to each 500-cc. unit of blood. This is especially noteworthy in case of blood to be carried by air transport, because the larger volumes of diluent add considerably to the volume and weight of fluid in each package per unit of blood. All the solutions have in common the use of sodium citrate as the anticoagulant and (after the first-named) varying concentrations of dextrose (glucose), which serves as a necessary substrate for the glycolytic enzymes in the cells. Sodium chloride is used in some solutions to maintain favorable osmotic conditions. Acidity of the solutions is a factor of importance in preparation of the fluids, as well as in their effect on cell preservation. Dextrose is caramelized if autoclaved with sodium

citrate in neutral or alkaline solution; consequently the British Transfusion Service added dextrose solution, separately sterilized, to each bottle after the blood had been drawn into sodium citrate solution. Acidification of the citrate-dextrose solution to pH 5 or 6 provides the advantage that the complete fluid can be sterilized in blood-collection bottles by autoclaving without caramelizing. A low pH of the final blood-diluent mixture (around 7) has an important effect during storage, apparently improving preservation of the cells by stabilizing the glycolytic enzyme system and minimizing particularly the changes of adenosine triphosphate. The adjustment of pH is accomplished in case of the Denstedt and Muether solutions by the addition of phosphate buffers, and in the case of the Loutit-Mollison's solution by the addition of citric acid or the substitution of disodium citrate for trisodium citrate.

Under the general designation of ACD, many modifications of the original Loutit-Mollison formula were subjected to thorough study and to extensive practical trials in civilian hospital blood banks, where some of the investigators had charge of large transfusion services. When the accumulating evidence of the superiority of the ACD solution appeared to be conclusive, recommendations were finally transmitted, in August 1944, to the Surgeons General of the Army and Navy and the United States Public Health Service for the use of the following formula: 1.33 per cent trisodium citrate ($Na_3C_6H_5 \cdot 2H_2O$), 0.47 per cent citric acid, and 3 per cent dextrose. Four parts of blood are added to one part of the solution. The pH of this solution is 5, and the pH of the diluted blood is 7 or 7.1.[1]

The efficacy of the recommended ACD solution and, for comparison, three other solutions in preserving the viability of erythrocytes in stored blood is shown in Figure 62, a chart based on data gathered by several groups of investigators who studied the post-transfusion survival of transfused blood cells by the radioactive iron isotope-labeling technic. Points on each curve indicate the percentage of transfused cells found surviving in the circulation of the recipient twenty-four to forty-eight hours after transfusion of bloods that had been collected and stored at 4° C., for the number of days indicated

[1] The search for further improvements in methods for the preservation of whole blood was still going on when the OSRD(CMR) program was brought to a close at the end of the war, with the investigators believing that they had not exhausted all the possibilities of finding ways to obtain a still longer period of preservation than was given by the ACD solution then in use. At the last meeting of the Subcommittee on Blood Substitutes in Washington on December 8, 1945, a modification of the ACD formula was recommended for a solution composed of trisodium citrate ($2H_2O$) (2.20 gm.), citric acid U.S.P. (0.80 gm.), and dextrose U.S.P. (2.45 gm.) in 100 cc. of water. This solution, of pH 5, is to be used in the proportion 15 cc. of fluid per 100 cc. of blood — generally 75 cc. of fluid per 500 cc. of blood. It gives equally good preservation of the cells as compared with the ACD solution, and affords the advantages of requiring a smaller volume of diluent, reducing the volume of fluid to be transported and administered per unit of blood cells, and making somewhat easier the processing of plasma drawn from the blood where this operation is carried out.

by the base line, in simple citrate solution (Curve 1), Alsever's solution of pH 7.3 (original formula) (Curve 2), a modified Alsever's solution of pH 6, used for the first shipments of blood to Europe (Curve 3), and the ACD solution described above (Curves 4 and 5). The horizontal dotted lines at 70 and 90 per cent represent respectively the survival levels accepted as indicating satisfactory and optimal preservation. Survival rates falling below
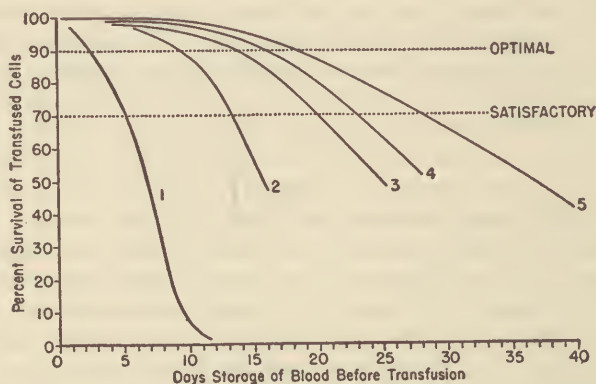

FIGURE 62. *Post-transfusion survival, determined by the radio-active iron isotope technic, of the red cells of bloods collected in sodium citrate solution (1), Alsever's solution, original formula (2), modified Alsever's solution of pH 6.0 (3), and ACD, or acid-citrate-dextrose solution (4 and 5).*

70 per cent indicate, for example, that preservation was less than satisfactory with sodium citrate solution after only five days. Curves 4 and 5, derived from separate sets of data, suggest a possible range of variation in the preservability of bloods collected from individual donors; such variation is suggested also by other data, gathered from in vitro studies. These curves indicate that good preservation of blood stored in ACD solution is maintained well beyond the twenty-one-day limit set by the military services for field use. Curve 3 indicates how preservation in the Alsever type of solution was greatly improved by the modification providing for greater acidity, of pH 6.

When the recommendation of the ACD solution was transmitted to the armed services through the Division of Medical Sciences, National Research Council, it was recommended also that provision be made for cooling the blood immediately after collection and for its continuous refrigeration during transportation and storage until use. The Navy, making plans for the long-distance shipment of blood to the Pacific area through tropical zones,

solved the problem of refrigeration quickly and admirably by the development of an expendable refrigerator box 5.9 cubic feet in size. This box, developed at the Naval Medical Research Institute, was constructed of plywood, with 4 inches of fiberglass insulation, with wire racks to hold two tiers of eight bottles of blood, and the accompanying apparatus for its administration. A refillable removable metal capsule in the center held 10 pounds of cracked ice as the refrigerant. When pre-chilled and filled with bottles of chilled blood, the box maintained the recommended internal temperature of 4 to 10° C. up to fifty hours, with ambient temperatures approximating 27° C. (85° F.). The ice containers could be renewed whenever necessary and the compact boxes easily stored in large refrigerators at each stopping place on long air-transport journeys. The total weight of the box, packed with sixteen bottles of blood, was 87 pounds. Thorough tests of the practicability of the refrigerator boxes and of the long-distance shipments were made by sending separate lots of blood (in ACD solution) from the United States to Pearl Harbor and back to Washington, where the bloods were tested by in vitro methods and by transfusion into patients. The sturdiness of the boxes was later demonstrated when they were dropped by parachute, without breakage of the bottles, to isolated medical units in the battle zones.

The increasing demand for whole blood on the war fronts is attested by reports on its use from the military services. Original estimates of the amount of whole blood needed were based on the expected use of around 1 pint of blood for four or five casualties. Actually the use commonly increased to 1 pint for two casualties, and in some areas of fighting — for example, in the Philippines — to 1½ pints per casualty. Meeting this demand was made possible by the establishment in 1944 of the joint program of the American Red Cross, Army, and Navy for the collection of Group O (universal donor) whole blood in the blood-donor centers where blood was being collected for the processing of plasma. The American Red Cross assumed responsibility for the procurement of the blood, under the supervision of Army and Navy representatives; the Army undertook the delivery of blood to the European Theater, and the Navy its delivery to the Pacific Theater. After the program was fully developed the supply of whole blood from donors in the United States proved adequate to cover the needs in all areas overseas, and made almost unnecessary the continued use of service-personnel donors.

The first blood shipped from the United States to the European Theater was collected in modified Alsever's solution, the preservative fluid that had then received the most complete study at the Army Medical School. Although the early shipments, begun August 21, 1944, were made without refrigeration from New York to France (but with it before shipment and immediately on arrival), provision was later made for refrigeration during transport, using boxes similar to those employed by the Navy for shipments to the Pacific. In April 1945, the Army also adopted the ACD solu-

tion for blood to be used in the European Theater. When this service was stopped on May 10, 1945, after the cessation of hostilities, a total of 201,105 bottles of blood were reported delivered overseas. In November 1944, the Navy began flying blood, collected in ACD solution and carried in the refrigerator boxes described above, from San Francisco to Guam, the distributing center for the Pacific area, and had delivered in this manner 177,784 bottles of blood when the war ended. The distance from San Francisco to Guam is 5700 miles, requiring thirty-one hours flying time, and from Guam to the Philippines 1000 miles, eight hours flying time.

This distribution of whole blood to distant areas had been widely acclaimed as one of the major advances in the medical practice of World War II, and represents one of the finest examples of the benefits of thorough collaboration between research workers and those who applied the results of their work. Reports on the use of this blood, which do not form a part of this history, will cover the largest groups of data on transfusions and their reactions in the history of blood-transfusion practice, and the medical experience gained therefrom will prove of inestimable value for the future guidance of civilian as well as military blood banks and transfusion services.